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ABSTRACT

This booklet contains a six-week series of laboratory investigations that may be used individually or in combination to complement other biology course materials or as an independent laboratory course in radiation biology. Contents include twelve activities dealing with radiation biology, five additional activities suitable for individual work, and four appendices which cover the handling of microorganisms, a discussion on radiation, how radioactive materials are produced, and how a Geiger-Muller counter is operated. (CS)

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BIOLOGICAL SCIENCES CURRICULUM STUDY

COMMITTEE ON INNOVATION IN LABORATORY INSTRUCTION

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## A Laboratory Block

# Radiation and Its Use in Biology

by

**WILLIAM V. MAYER**

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## LABORATORY BLOCKS FOR BIOLOGY

The Laboratory Blocks are a series of books, each of which provides for the investigation in depth of a specific topic in biology. The series has been developed by the Committee on Innovation in Laboratory Instruction of the Biological Sciences Curriculum Study and has been supported by the National Science Foundation.


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## FOREWORD

The Biological Sciences Curriculum Study, supported by the National Science Foundation, was initiated in 1958, with the charge to improve biological education at all levels. For this purpose, instructional materials have been designed that reflect the modern content of biology without neglecting the wisdom of earlier scholars and authors. The basic approach of the Biological Sciences Curriculum Study has been to emphasize independent laboratory investigation and inquiry as a means of acquiring significant knowledge in science. Materials produced by the BSCS utilize a variety of approaches to the study of biology, for there does not seem to be a single best way of presenting biology to the diversity of students studying the subject. The BSCS has produced materials designed for all students regardless of aptitudes or career goals. These materials have been successfully mastered at a level of student sophistication that was heretofore thought completely unrealistic.

Taken together, the BSCS materials comprise a series of balanced and enriched programs, not simply a set of independent publications and audio-visual materials. The BSCS has developed a wide variety of materials for use at both high school and college levels, including three different approaches to introductory biology for secondary school students, a program for the academically unsuccessful, a second course in biology, *Research Problems in Biology*, a variety of pamphlets on specific biological subjects, programmed materials, a *Biology Teachers' Handbook*, bulletins, special publications, Newsletters, Single Topic Inquiry Films, Inquiry Slides, and other material.

Innovation has been a keynote of the BSCS, and among the innovations has been the investigation of ways to encourage use of the laboratory as a means of promoting student inquiry and self-reliance. One of the ways of emphasizing the laboratory as a productive learning experience has been the series of Laboratory Blocks produced under the direction of the BSCS Committee on Innovation in Laboratory Instruction. Each Block is a program requiring approximately six weeks of time, during which the student investigates a specific biological topic in depth. Any one of the Laboratory Blocks may be used in conjunction with a wide variety of course materials to replace, to supplement, to complement, to enrich, or to augment a specific component of the curriculum as necessary.

The Laboratory Block program has resulted in a group of books written either by a single author or, at most, a small group of specialists in a given field. The preliminary books were tested by BSCS Project Associates in a special BSCS testing laboratory at the University of Texas before undergoing trial use in a series of classrooms across the country. Based on this extensive experience, the books were thoroughly revised before release for general use. The present volume is an example of one of these Laboratory Blocks, and the Preface, which is addressed to the student, illustrates the rationale behind the development of these Blocks.

The BSCS feels biological education is a continuum, from kindergarten through university graduate school, and is attempting to devise a nonrepetitive series of biological experiences to be used at the most appropriate level in a given program. The Laboratory Blocks constitute one kind of materials that can be used at a wide variety of instructional levels and in a large number of different situations. Adaptability and flexibility make this varied use not only possible but academically profitable within a particular curricular sequence. More detailed information on the philosophy and use of Laboratory Blocks is contained in BSCS Special Publication No. 5, *Laboratory Blocks in Teaching Biology*, available free upon request to the Director of the BSCS.

With each new generation, our fund of scientific knowledge increases fivefold. This remarkable growth indicates in part that only selected areas can be covered in depth at a given point in time. Laboratory Blocks provide this in-depth coverage of a specific topic; they have been used as individual items in given parts of a curriculum, or several have been combined in various permutations to create entire laboratory-centered, inquiry-oriented courses in biology.

The philosophy that has developed around the BSCS Laboratory Blocks evolved from stressing the importance of laboratory work in the teaching of biology. Dr. Bentley Glass, former Chairman of the BSCS, suggested that if

one were to design a continuous experience in the laboratory, it would be possible to have the laboratory work carry an entire topic within a course and give the student significant and extensive laboratory experience that could not be gained by laboratory segments scattered periodically throughout a course. The Laboratory Blocks are one mechanism for implementing this approach to laboratory activities.

Hundreds of the nation's biological scientists, educators, and teachers have worked diligently on BSCS programs. Continued success and growth of the program is largely due to these dedicated individuals and to those who have given unselfishly of their opinions and experience in the classroom.

One of the strengths of the BSCS program has been the incorporation of reactions gained by the use of the materials in their betterment. This feedback has contributed constantly to the improvement of existing materials and provided ideas for still newer ones. Your comments and suggestions are invited. Send them, together with requests for information about other BSCS projects intended to improve biological education, to the Director at the address below.

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During the next six weeks we expect you to work and to think like a scientist or research worker. Your actions in the laboratory or while doing homework should be directed by logical thinking. Each step of your investigations should be governed by the basic questions: *How? What? Where?* and *Why?* The answers you get will be precisely as correct as your combination of background knowledge, logical thinking, and accurate experimentation let them be.

The scientist does not know all the answers; he is seeking to learn the answers just as you are. He has spent many years reading widely on the subject of his interest so that his background knowledge is extensive. By carefully studying the work of others and comparing it with his own, he can make a critical evaluation of our present state of knowledge. Then, carefully, he plans experiments which will provide observations, or data, that can be interpreted and that may add new knowledge.

Since you do not have the background of reading and experience in scientific research that would let you plan the material in this Laboratory Block, you are asked to take our presentation on faith—up to a certain point. The scientist would decide what to study and plan how to study it. However, you will need to follow the choices and procedural instructions that we will give you in this book. Both you and the scientist obtain data, and from this point on your work will be the same—analyzing the data and interpreting the results. At this point in research the scientist would make a decision on what to do next—repeat the experiment, discard it, or plan the logical next step.

The first experiments could end in failure for either you or the scientist. The scientist sometimes learns more from his failures than from his successes. But he does make discoveries; that is, he may find information to indicate that his working hypothesis is correct or that it is not correct, or he may find something he had not anticipated. So will you.

The exercises that follow have been designed to guide you in making some discoveries—some that have already been made by others and perhaps some that have not. The procedure given in this Laboratory Block is one that a scientist might follow if he were studying the same problem. Therefore, during the next six weeks we expect you to work like a scientist. And after that, who knows? You may want to become a scientist.

*The Committee on Innovation*
*in Laboratory Instruction*

## ACKNOWLEDGMENTS

A work such as this cannot be credited to one man alone. While one man is ultimately responsible for the assemblage, order, style, and mistakes that may occur, this Block is a cooperative effort that has involved a large number of individuals, from the members of the BSCS Committee on Innovation in Laboratory Instruction to those who tested the experimental Block in actual classroom situations.

Three Project Associates of the BSCS Testing Laboratory at the University of Texas tested and evaluated the various inquiries and recommended modifications prior to the appearance of the experimental edition used in the general BSCS trial program. To them, the author is most grateful. They are: Dr. Marjorie Behringer, Department of Biology, University of North Dakota, Grand Forks, North Dakota; Dr. David L. Lehman, 709 Leonard, Austin, Texas; and Dr. H. Edwin Steiner, University of South Florida, Tampa Campus, Tampa, Florida. Mrs. Mary Anne Hunter, Administrative Secretary at the BSCS Austin Testing Laboratory, greatly facilitated work in conjunction with this Block.

Thirteen teachers tested the Radiation Block in their classes and provided valuable feedback that contributed to the book in its present form. Without their aid, this volume would be far less useful in actual classroom situations. They are: Bob Anderson, Gunn Senior High School, Palo Alto, California; Andrew Browne, Los Altos High School, Los Altos, California; Rex Carr, Shawnee Mission South High School, Shawnee Mission, Kansas; Larry Hull, Cubberley Senior High School, Palo Alto, California; C. T. Lange, Clayton High School, Clayton, Missouri; Dr. Leonard Molotsky, Shawnee Mission High School District, Shawnee Mission, Kansas; Roy Rosendahl, Homestead High School, Sunnyvale, California; Frank W. Smith, Jr., Los Altos High School, Los Altos, California; Nancy E. Stees, Kennett Junior-Senior High School, Kennett Square, Pennsylvania; Larry Webster, Shawnee Mission West High School, Shawnee Mission, Kansas; Marlin Welch, Shawnee Mission East High School, Shawnee Mission, Kansas; Lilly Wing, Ravenswood High School, East Palo Alto, California; and Harry Wong, Menlo-Atherton High School, Atherton, California.

Four individuals deserve special mention in conjunction with the preparation of this Laboratory Block. Dr. Walter Chavin of the Department of Biology of Wayne State University in Detroit, Michigan used the experimental edition in his jointly sponsored NSF-AEC Summer Institute in Radiation Biology. The feedback and contributions from the teacher-participants in this institute were of great help,and only space limitations prohibit listing each by name. The author is grateful to Dr. Chavin for the rigorous testing his institute participants gave the experimental edition.

Mr. Patrick Balch of the Yuba City High School, Yuba City, California critically reviewed the final draft of this manuscript and made numerous helpful suggestions regarding organization and emphasis on inquiry which greatly improved the teachability of the Block.

Dr. H. Edwin Steiner was the Project Associate most closely involved with the materials of this Laboratory Block. He was inordinately helpful, patient, constructively critical and aided immeasurably in assessing the practical value of the inquiries herein.

Mr. Clarence T. Lange of Clayton High School in Clayton, Missouri gave the author exceptionally helpful feedback from the use of the experimental edition. He was extremely generous in supplying class results from his usage of the experimental book and gave generously of his own thoughts, materials, and experience garnered from years of classroom teaching involving radiation biology.

To those many others whose suggestions, comments, and freely offered materials have been incorporated into this work, the author extends his thanks and the gratitude of students who will profit by these contributions.

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# INTRODUCTION *WHAT IS RADIATION?*

You turn over on a Florida beach so your back will get a more uniform tan. At the end of a hard day's skiing in Colorado you warm yourself in front of the blazing logs in the lodge fireplace. In New York City, you watch the Rose Bowl game from California on television. In Seattle, your radio hammers out the "Top 40" for the week. Your doctor takes a chest x ray during a routine physical. What do these events all have in common?

You are already familiar with most of the following words:

| | | |
|---|---|---|
| radio | radiation | radium |
| radiant | radioactivity | radiator |
| radius | radar | irradiate |

What do these words have in common?

The examples in the first paragraph all involve radiation of some kind. The library will provide a source of references so that you can determine what kind of radiation is involved in each case. In the second paragraph, all the words contain the same three letters (rad) and most the same four (radi). The base word for all of these is the Latin *radius*, which means ray. Thus, each of these words has something to do with a ray. By using the dictionary, you can determine this relationship. Man in space is exposed to cosmic rays, and man on earth to sun's rays. With what other kinds of rays are you familiar?

The sun's rays are the ones most frequently mentioned. The reason radiation from the sun is most familiar is that it can be seen daily as visible light. Other types of radiation are as common but not so well known because they are invisible to the naked eye. Our own experience with sunlight forms a basis for understanding some of the effects of radiation. The sun's rays provide us with the visible light by which we see, the comforting warmth when we lie on the beach, and our suntans. We can artificially duplicate some of these effects by the substitution of electric lights for the sun's rays, infrared lamps for the sun's heat, and ultraviolet lamps for the sun's tanning effect. Why do we need three different types of electric lamps to duplicate some of the effects of solar radiation? A simple and obvious answer is that there is not simply one kind of ray coming from the sun, but several different kinds. If, for example, we pass the apparently colorless sunlight through a prism, it is broken up into a rainbow of colors ranging from violet through red (Figure 1).

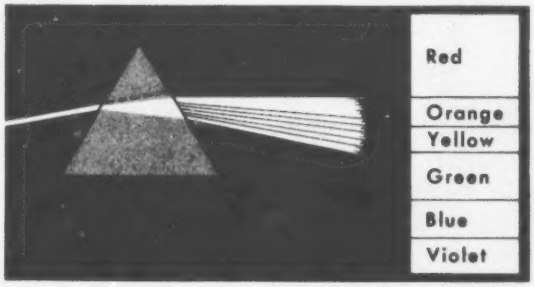



Figure 1. When a beam of white light passes through a prism, the shortest light rays bend the most and are found in the violet end of the spectrum. The longest rays are bent the least and are found in the red end of the spectrum.

The formation of this spectrum of colors is understandable if the assumption is made that the various parts that make up sunlight have different wavelengths that are each bent at a slightly different angle. If you think of light as traveling in waves somewhat like the waves of water, wavelength is the distance from the crest of one wave to that of the next (Figure 2).

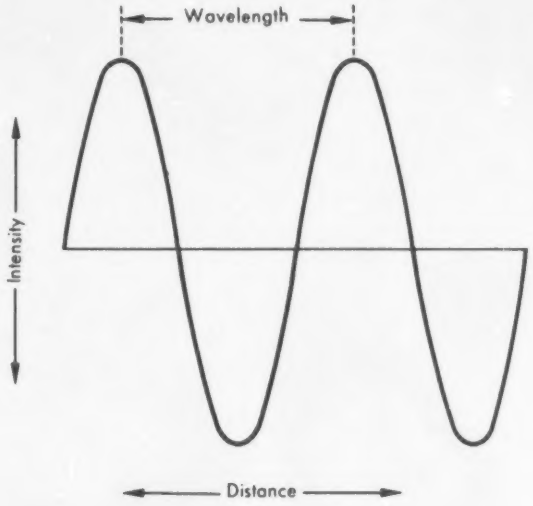



Figure 2. Electromagnetic radiation travels in waves. Wavelength is measured from the crest of one wave to the crest of the next. Wavelength may vary from fractions of a millionth of an inch to several miles.

According to the wave theory of light, the higher the wave, the greater the intensity of light. These light waves are similar to electrical waves passing along power lines and to heat waves radiating from hot surfaces.

While the wave theory of light is useful in working with certain physical phenomena, such as refraction, there is another theory of light, called the quantum theory, that describes light as being composed of small quantities of energy (quanta or photons). According to this theory, it is the number of photons per unit of time that influences the intensity of light. Thus, a very bright light shining for a short period of time could transmit the same number of photons as a dimmer light shining over a longer period.

While the quantum theory of light is useful in studying the interaction of light and matter, the wave theory provides us a better introduction to the energy carried by all electromagnetic radiations. Electromagnetic radiation is energy in the form of rays produced by the increase in speed of an electrical charge. We can identify various kinds of radiant energy by wavelength. The length of a light wave is measured in angstrom units (Å). Ten angstrom units are equal to 1 millimicron or 0.00000001 centimeters, usually expressed as $10^{-8}$ centimeters. The sunlight we see ranges between 3800 and 7600 Å in wavelength. Wavelengths of less than 3800 Å include ultraviolet light, x rays, gamma rays and cosmic rays, each smaller in wavelength than the other. Above 7600 Å, we deal with wavelengths for infrared, TV, radio, and electric waves of various kinds. Radiant energy can thus be described as extending from the visible spectrum on both ends to form what is known as the electromagnetic spectrum whose parts are identified by their wavelengths (Figure 3).

Another advantage of identifying radiant energy by its wavelength is the possibility of reproducing precisely the required part of the electromagnetic spectrum. Using visible light as an example, a certain shade of green can be identified as being of 5156 Å, or a particular shade of red can be described as 6600 Å. The region of the spectrum around 2850 Å is that which causes reddening of the skin and sunburn. What does this information suggest must be one of the properties of oils and other substances used as sunburn preventatives?

Electric heaters or steam radiators radiate heat at wavelengths greater than 7600 Å. Television and radio sets receive radiation in that part of the electromagnetic spectrum between 1 and 500 meters. Radiant energy of different wavelengths has different properties. One of these properties is penetration. It is this property that we need to understand to protect ourselves from various kinds of electromagnetic radiation. We can be shielded very easily from the sun. A thin piece of black paper, for example, will cut off all visible light, but you can go into a closet and close the door and still your portable transistor radio will play, indicating that radio waves are not stopped by the thickness of the closet door. You can devise numerous experiments indicating what wavelengths are transmitted through ordinary window glass or water or, by using your transistor radio, design containers that will stop the passage of radio waves.

We will, in using this Laboratory Block, be concerned with radiant energy beyond that of the visible spectrum. We will deal with radiant energy primarily of small wavelengths from ultraviolet light to gamma rays and including atomic particles.

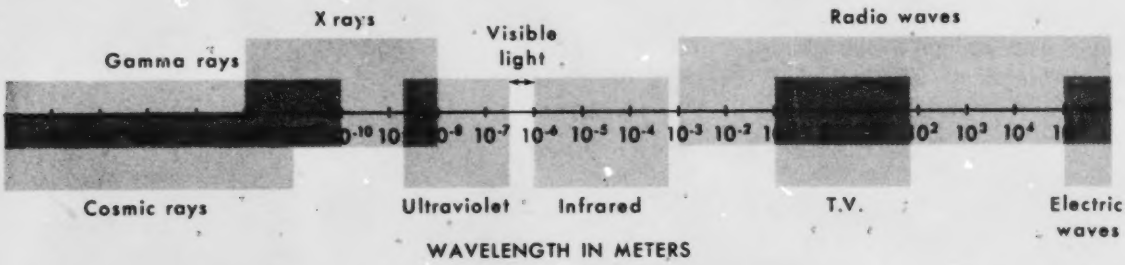



Figure 3. The electromagnetic spectrum is a continuous band of radiation. The physical nature of the radiation is the same throughout the whole range except for frequency and wavelength. The names of the sections are somewhat arbitrary, and they overlap. Radio waves result from electrons moving in conductors, infrared from hot objects, visible light from very hot objects, ultraviolet from arcs and gas discharges, x rays from electrons striking a target, and gamma rays from nuclei of radioactive atoms.

But first a word about safety. Everyday life is full of hazards. One may be killed or injured crossing the street, driving an automobile, falling down stairs, tripping over an untied shoestring, slipping in the bathtub, cutting oneself with a knife, or in any number of ways. Life is thus a hazardous situation, but we reduce hazards with ordinary caution and precautions. We look both ways before we cross the street. We do not step in front of a speeding car. As careful drivers, we keep our automobiles under control. We use handrails and do not run downstairs; we behave in ways that minimize the possibility of falling. And we tend, for the most part, to notice where accidents can occur and to act to prevent them.

In the laboratory, we are accustomed also to hazards. There are Bunsen burners, beakers of boiling water, acids, knives, pins, needles, and many other potential sources of danger. However, it is a very rare day in a laboratory when someone is injured, and then usually only when he or she fails to observe the normal laboratory precautions that would minimize such risks. Just as we can handle boiling water and caustic chemicals in the laboratory without danger, so can we handle radioactive materials safely. With radioactive materials, however, more care is required because the amounts are very small and their radiation can neither be seen, felt, smelled, tasted, nor heard. It is because this radiation is not evident to our senses that we think of it as mysterious and dangerous. Also, the fact that the effects of radiation are not immediately visible may give one a false sense of security. Various periods of time ranging from hours to generations may be required before the effects of radiation can be noted. However, just as we handle microbes in the biology laboratory when they cannot be seen, so by a series of somewhat similar techniques, radionuclides (radioactive chemicals) can be handled, transferred, and their effects observed as safely as any other laboratory material *if the proper precautions are used*. The specific techniques that are to be followed in dealing with radionuclides and radiation in general will be described where and when they are useful in the following inquiries. *Strict attention must be paid to the directions in the Laboratory Block and the instructions given by the teacher must be followed exactly*! Play safe with sources of radiation and you will have a tool that will reveal much about biological systems that could not be understood by any other method. Careless use, however, makes radiation not only unusable as a biological tool but exposes the investigator and the rest of the class to unnecessary hazards. Reasonable care and precautions are all that is necessary to use radiation to investigate biological phenomena with complete safety.

# INQUIRY I *HOW ARE MICROORGANISMS AFFECTED BY IRRADIATION?*

In this inquiry we will use radiant energy very close to the violet end of the visible light spectrum. We will use ultraviolet light which, while not seen by human eyes, can be perceived by insects such as the fruit fly, *Drosophila*. The wavelength perceived by *Drosophila* is roughly 3660 Å. Ants and many other insects,as well as persons with aphakic (lensless) eyes, can also see with this wavelength of light. If you could see ultraviolet light, in what ways would such an addition to your visual spectrum affect your interpretation of your environment? While you are not to look directly at the ultraviolet lamp, you will know when it is off and when it is on because you will notice a certain amount of visible radiation. However, this is not ultraviolet light but is visible light produced because the ultraviolet lamp is not 100 percent efficient in the production of pure UV light.

This first inquiry is designed to supply data from which you can determine some effects of irradiation on a particular cell population. Because of the easy availability of ultraviolet light, it will be used as a radiation source in this inquiry. The observations you make with the ultraviolet light will be useful when you use other types of radiation, even though the action of ultraviolet radiation is technically different from that of x rays or gamma rays.

### CAUTION

The ultraviolet light source should be well shielded and turned on only for the actual purpose of irradiating the specimens. Materials should be moved in and out of the radiation field with tongs. *Do not expose hands to the ultraviolet light. Do not look directly into the lamp as its radiation is particularly harmful to the retinal tissue of the eye.*

### MATERIAL (per group)

Agar, potato dextrose, 150 ml
Autoclave or pressure cooker
Cotton, sterile
Flask, Erlenmeyer, 250 ml (2)
Flask, Erlenmeyer, 1000 ml
Hydrochloric acid, 0.1*N*, 2 ml
Lamp, ultraviolet (2537 Å)
Marking pen, felt
Paper, black (marked off in 1 cm squares for use in counting colonies)
Paper, *p*H
Petri dishes, (8)
Pipette (with bulb), 1 ml in 0.1 ml units
Rod, glass (bent like a hockey stick to use as spreader)
Tongs
Water, distilled, 200 ml
Yeast, dry, 1 g

### PROCEDURE

This inquiry assumes you are familiar with the handling of microorganisms. If your laboratory work so far has included microbiological techniques, you may wish only casually to refer to Appendix I ("How Are Microorganisms Handled?") to refresh yourself as to the procedures to be used. If you have not yet mastered microbiological techniques, this appendix should be read with care as microorganisms are excellent for radiation studies and will be used again in this Block.

1. Follow the directions on the package for the preparation of potato dextrose agar. The medium works best if adjusted to a *p*H of 3.5 by the addition of 0.1*N* HCl one drop at a time. Check the *p*H by means of *p*H paper. Sterilize the medium in the autoclave or pressure cooker at 15 pounds pressure for 15 minutes. A 150 ml flask of agar is sufficient for the 8 petri dishes. Sterilize the petri dishes in a hot air oven at 175°C for an hour. Pour the melted potato dextrose agar into the 8 sterile dishes using careful aseptic techniques. Label the petri dishes with a felt marking pen indicating your name, the date, and later add the length of time each dish is to be irradiated.

2. Prepare a yeast solution with a concentration of $10^{-7}$ grams per milliliter as follows:

   a. Weigh out 1.0 gram of dry yeast.
   b. Pour the dry yeast into 1000 ml of sterile, distilled water. Plug the container with cotton and swirl the contents to mix thoroughly.
   c. Transfer 1.0 ml of this first solution to 99 ml of sterile, distilled water. Again, mix well.

d. From this second yeast solution, transfer 1.0 ml to 99 ml of sterile, distilled water and mix well. This last solution is a $10^{-7}$ g/ml concentration.

3. Pipette by bulb 0.1 ml of the $10^{-7}$ g/ml yeast solution onto the agar in each of the autoclaved petri dishes. One person can inoculate several agar plates (up to 10) at one time with one pipette (use a 1 ml pipette marked in 0.1 ml divisions) by filling the pipette then releasing 0.1 ml of the yeast solution into each petri dish. The yeast solution can be spread over the surface by the use of a sterile glass rod bent to resemble a hockey stick. Be sure to distribute the yeast evenly over the surface of agar but try not to let the solution reach the edge of the petri dish.

4. Fasten the ultraviolet lamp 10 cm above the table top.

5. By means of tongs, place one of the petri dishes, with cover removed, containing the 0.1 ml of the yeast suspension under the lamp for exactly five seconds. *Do not keep your hands under the lamp. Do not look at the light source.*

6. After the five-second exposure, remove the petri dish with tongs and replace the cover.

7. Repeat step 5 and irradiate other samples (one at a time) for periods of 15 seconds, 30 seconds, 60 seconds, 2 minutes, 5 minutes, and 10 minutes.

8. One of the 8 prepared petri dishes of 0.1 ml of yeast suspension has not been exposed to ultraviolet radiation. What is the purpose of this dish?

9. Incubate all inoculated plates in the dark at about 25°C for 48 hours. The cultures grow better at 37°C in an incubator if available.

10. Count the number of colonies formed from the untreated cells. Since the colonies are light in color, place a black piece of paper marked off in centimeter squares with a white pencil under the petri dish. The paper squares allow for more accurate counting. Count the number of colonies present in the untreated culture. Use this number to represent 100 percent survival.

11. Count the number of colonies in each treated culture. Use this figure to represent the number of survivors in each petri dish. Compare this figure with that for the untreated culture. Calculate the percentage of survivors for each of the treated cultures. One hundred percent minus this percentage indicates the percent affected by the radiation treatment.

12. Prepare a graph with the length of time of irradiation on the horizontal axis and the percent of the cells inactivated on the vertical axis. Of course, at 0 time none was affected. The greatest exposure—10 minutes—may affect all the cells. In plotting the time on the horizontal axis, remember that each square on the graph paper represents a given unit of measurement in seconds or minutes. Thus, the 5-minute and 10-minute points will be much further from each other than the 15-second and 30-second points. After marking appropriate points on the graph paper representing the percent of the cells inactivated per unit of time, draw a smooth line connecting the points plotted on the graph. If time allows, plot the survival curve using as the vertical axis the percent of growing cells. How do these data compare with those for inactivated cells?

## Analysis of Data

In general what effect did irradiation have on individual yeast cells? Now, referring to your data, at what point, if any, were all the cells inactivated by irradiation? Between what two times was the effect of the irradiation greatest? What is the significance of the shape of the line on the graph paper? What does it indicate concerning the power of radiation? At approximately what time of exposure would one-half of the cells have been inactivated? What was the fate of those cells which did not grow on the irradiated plates? Were they necessarily dead? What other explanation besides death would explain the data? Given the data obtained, could the effect of radiation on other species be predicted? Would the amount of irradiation required to inactivate half the cells of a new species be about the same as that in this inquiry? Would the general shape of the graph obtained from this inquiry be about the same if a different species were used? What possible effects would the irradiation have had on the still-living organisms in this inquiry? Having observed the effects of ultraviolet radiation on a microorganism, suggest what practical applications might be made of such irradiation.

## Problems

As time allows, certain groups may use microorganisms other than yeast and prepare data on dosages required to inactivate 50 percent of the individuals of different species of

microorganisms, such as *Serratia marcescens*, *Sarcina lutea*, or *Penicillium*.

The effect of ultraviolet radiation on streak-plate and pour-plate cultures may be contrasted. Further data may be obtained by the introduction of glass, a film of water, or other filters between the ultraviolet source and the microorganisms. Such experiments will give some indication of the penetrating power of the ultraviolet rays through the various media by their effect on microorganisms.

Similar investigations may be performed using either different types of radiation or organisms. For example, earthworms exposed to sunlight readily show the effects of solar radiation. If the data obtained from experiments of this nature are to be attributed solely to the effect of sunlight, what are the factors that need to be controlled?

# INQUIRY II *CAN THE EFFECTS OF IRRADIATION BE MODIFIED?*

In the first Inquiry, you determined an effect of ultraviolet radiation on microorganisms. For these organisms, it is possible to think of the ultraviolet light as a "death ray." It is also possible for gamma rays, x rays, and other parts of the electromagnetic spectrum to act, in effect, as death rays. Except that they cannot be shot dramatically from a pistol and that their effects are not as instantaneous as in science fiction stories, these rays do affect living organisms.

Not only is science fiction full of reference to death rays, but even to antideath rays that can turn away or otherwise render harmless the effects of radiation. Death rays and antideath rays were products of the imagination of science fiction writers just as were voyages to the moon. While the United States has now placed men on the moon, we have not yet perfected a death ray similar to that used by Flash Gordon. We have, however, come to understand the lethal properties of radiation. Similarly, though we have not perfected an antideath ray, scientists have investigated radiation itself as a means of combating radiation effects. In this Inquiry, we will investigate a process known as photoreactivation. We will determine what effect visible light has on cells previously exposed to ultraviolet light.

As you remember from Inquiry I, ultraviolet radiation can damage and kill living cells. Is there some way that this damage can be lessened or prevented? For the purposes of this Inquiry, we will use yeast and ultraviolet radiation as in Inquiry I. We will add the additional step of exposing the yeast cells, after they have been subjected to ultraviolet irradiation, to various wavelengths of visible light and compare their effects.

MATERIAL (per group)

Agar, potato dextrose, 75 ml
Autoclave or pressure cooker
Cotton, sterile
Flask, Erlenmeyer, 250 ml (2)
Flask, Erlenmeyer, 1000 ml
Forceps
Hydrochloric acid, 0.1*N*, 2 ml
Lamp, ultraviolet (2537 Å)
Light sources (2—one for white light, one for blue light)
Marking pen, felt
Paper, black (marked in 1 cm squares for use in colony counting)
Paper, *p*H
Petri dishes, (4)
Pipette (with bulb), 1 ml in 0.1 ml units
Rod, glass (bent like a hockey stick to use as spreader)
Water, distilled, 200 ml
Yeast, dry, 1 g

PROCEDURE

1. This Inquiry begins with the same techniques as in Inquiry I. Follow steps 1, 2, and 3 of the procedure for Inquiry I. The 75 ml of agar will be sufficient for the four petri dishes per group used in this Inquiry.

2. Label with the felt marking pen the petri dishes before adding the yeast solution. Label dish 1 "no irradiation," dish 2 "UV light," dish 3 "UV light + white light," dish 4 "UV light + blue light."

3. Before exposing dishes 2, 3, and 4 to the UV light, determine the length of time the dishes are to be exposed to irradiation. Refer to the results of Inquiry I for data by which to make this determination.

4. Remove the cover from petri dish 2 and, by means of forceps, place it under the ultraviolet lamp for the period of time determined in 3 above. *Do not keep your hands under the lamp. Do not look at the light source.*

5. After the exposure, remove the petri dish with the forceps and replace the cover over the yeast suspension.

6. Repeat step 4 with petri dish 3. After the irradiation, replace the cover and immediately place this dish 10 to 20 cm from a strong, white light for a period of 30 minutes. Be careful that the culture does not get overheated.

7. Irradiate dish 4 as for dish 2 and, after irradiation, replace the cover and immediately place this dish 10 to 20 cm under a source of blue light for a period of 30 minutes.

8. Place all four plates together in the dark at about 25°C for 48 hours. The cultures grow better at 37°C in an incubator if available.

9. After 48 hours, count the number of colonies as indicated in step 10 of Inquiry I.
10. Record the data and examine them. If desired, these data can be graphed.

### Analysis of Data

What is the significance of these data? In your data analysis, compare the difference in the number of colonies between dishes 1 and 2 to determine the effect of irradiation. How does dish 2 compare with dishes 3 and 4 regarding the number of colonies present? How do you account for these differences?

The exposure to visible light after irradiation results in photoreactivation. How is photoreactivation a kind of antideath ray? Support your statement with reference to the data of this Inquiry.

### Problems

If the plates had been exposed to the white or blue light for a longer period of time, would you expect more or fewer colonies to be present? What would have been the effect of exposure to red light or green light or to some color other than blue or white? Would a longer exposure to a less intense light source have been more or less effective than a short exposure to a high intensity light source? Investigations designed by you can provide data to answer these questions.

# INTRODUCTION *TO RADIONUCLIDE USE—CAUTIONS AND PRECAUTIONS*

In the remainder of this Block we will be using radionuclides as radiation sources. Useful background material on radiation is to be found in Appendix II, which may be reviewed at this time. As the energy available in various forms of radiation is inversely proportional to their wavelengths, gamma rays have more energy than the ultraviolet light used in Inquiries I and II. Similarly, the tissue-penetrating power of radiation is directly proportional to the energy of its wavelength. This means that we will be dealing with radiation of higher energy and deeper potential penetration than the ultraviolet light of Inquiries I and II. Therefore, radionuclides should be handled with care, and the directions in the Laboratory Block and those given by your teacher should be followed exactly.

The Inquiries that use radionuclides demand thoughtful preparation and careful techniques. When we handle strong acids or heated materials, we are careful because we know that careless handling of either will produce an immediate and dramatic reaction. However, radionuclides carelessly handled do not produce either an immediate or a dramatic reaction. One could be exposed for a considerable period of time before there would be any evidences of the effects of radionuclides on one's health. Use of radionuclides requires constant care on your part in order that unseen radiation does not contaminate either the laboratory or individuals. Properly handled, radionuclides are no more dangerous than properly handled boiling water or lye solution.

The following general precautions should be observed when dealing with all radionuclides.

1. All radionuclides must be handled carefully, avoiding unnecessary exposure to ionizing radiation. Good habits of safe handling of materials *must* be formed early. Radioactive materials are never handled directly. They are handled with forceps or tongs. Solutions are never pipetted by applying suction with the mouth. Mechanical or bulb type pipettes are always used instead. Wherever vaporization of radioactive materials is required, this operation should be performed in a fume hood. The room should always be well ventilated.
2. All inquiries must be carefully planned in advance and you should understand completely what is to be done at all times. Experiments should be run through without use of radioactive chemicals before using radioactive nuclides in order to perfect techniques and to reduce possibilities of error and loss of radioactive materials.
3. Avoid any direct contact with radioactive materials. They should not touch the skin or be taken into the body either through the mouth or by breathing. Nothing must be placed in the mouth or on the lips in the laboratory. This includes food, beverages, cigarettes, cosmetics, gum, or any other materials.
4. The laboratory and equipment must both be monitored. That is, possible radioactivity should be checked with a Geiger-Muller (G-M) counter, which you will learn to use later, to make sure that both the laboratory and equipment are free of contamination. This must be done during and after each Inquiry and at regular intervals in addition. At the conclusion of the Inquiry, hands are to be washed carefully and placed in front of the Geiger-Muller tube window (not against it or over the tube itself) to determine if radioactive contamination is being carried away on the hands.
5. Protective clothing prevents contamination of the skin or personal clothing. Rubber gloves or plastic disposable ones are always used to cover the hands when working with radioactive material or potentially contaminated equipment. Protective clothing, such as a laboratory coat or apron, should be used to cover personal clothing when handling radioactive materials.
6. The most frequent cause of contamination is spillage. It is easier to prevent spillage than to decontaminate afterwards. Spills can be avoided by placing smaller open containers into larger ones,

such as beakers, or into a hole drilled in a flat block of wood that is not easily upset. Possible spills should be confined by working on a tray or a large shallow pan lined with an absorbent material with a water-repellent backing. Such absorbent material is usually available in large rolls and can be obtained from local paper companies. Disposable diapers or newspapers backed with a layer of oiled or waxed paper may be used instead to prevent the spill from contaminating the working surface. Cover the working area where radionuclides are handled with several layers of newspaper backed with plastic wrap or wax paper. At the end of the Inquiry, these are to be discarded in the radioactive waste container (see 10 below). The laboratory table, sink, and equipment are to be free of contamination at the end of the Inquiry. Proper procedure not only prevents exposure of other people using the laboratory, but also prevents contamination that will interfere with the accurate usage of the Geiger-Muller counter in future Inquiries.

7. All radioactive materials must be labeled so that they may be recognized. Glassware and equipment that have been used and decontaminated, if found still to have traces of radioactivity, should be labeled radioactive and separated from general use. As a common practice, it is best to segregate all apparatus used with radioactive materials, whether it has been decontaminated or not. The area in which work with radiation is done must be marked with the magenta three-bladed symbol on the yellow background as prescribed by the Atomic Energy Commission (Figure 4). All containers of radioactive materials must likewise be marked.
8. All radioactive materials must be kept under lock and key when the responsible individual is not in the laboratory.
9. The working area should never be cluttered. Unnecessary equipment should be removed from the working area in which it might be contaminated.
10. Radioactive waste should be collected and plainly labeled radioactive. Quantities of soluble material obtained under general license can generally be discharged into the sanitary sink if diluted with large volumes of water. All other materials should be placed in a special covered container clearly marked "Radioactive Waste" for later disposal. Into this container place all exposed soil, paper, sticks, bottles, and other supplies that are used in these Inquiries. Never discard radioactive wastes in any container but that specially marked for the purpose.

Figure 4. This symbol indicates the presence of radioactive materials. The three-bladed symbol is magenta on a yellow background and is prescribed by the Atomic Energy Commission.

11. Despite all precautions, accidents sometimes happen and decontamination procedures are necessary. Knowing this, you will find it easier to decontaminate an area if the working surfaces are impermeable to aqueous solutions. This can be accomplished by coating the working surface with wax. If, despite all precautions, an area or part thereof becomes contaminated with the radioactive material, the following procedure is to be used:
    a. For spills of dry material, apply sticky paper, such as masking tape or cellophane tape to pick up the material. Do not try to sweep it up. Sweeping usually spreads the contamination without removing it.
    b. With liquids, absorbent papers can be used to blot up as much contamination as possible. They should be discarded in the radioactive waste can.
    c. The contaminated surfaces can then be further cleaned with liquid cleaners. Your teacher has a list of general reagents for decontamination and procedures for handling specific types of contamination.

## Basic Materials

*In order that the above general precautions can be observed, the following materials must be available and used in every Inquiry in which radionuclides are handled:*

Clothing, protective, such as laboratory coat or apron

Decontamination equipment

Forceps or tongs for handling radionuclides

Gloves, rubber or plastic, disposable

Monitoring equipment (Geiger-Muller counter)

Radiation labels, including a large warning sign for the work area, and small warning stickers and tape to label appropriate containers and materials

Radioactive waste container

Spillage control materials such as larger containers for smaller open containers, large trays or shallow pans, absorbent material with water-repellent backing, such as newspaper backed with plastic wrap or wax paper

This is a master list for each laboratory in which radioactive materials are to be used. The introductory Inquiries using radionuclides list some of this material, but later ones do not in order to clarify the essential materials required for the individual experimental design. However, it must be kept in mind that *the above materials must be available for each of the Inquiries using radionuclides whether listed or not.*

# INQUIRY III *HOW CAN YOU SEE RADIATION?*

In the Introduction it was pointed out that visible light is but a small part of the electromagnetic spectrum. In Inquiries I and II you were using light that was specifically stated not to be visible to the unaided eye but whose effects could be noted. The individual yeast cells used in Inquiries I and II were also invisible to the unaided eye, yet they could be handled and later their presence was made known by their colonial growth. In these examples the invisible could be observed by its effects, and initially invisible objects handled and later seen.

When you drive your car across a shiny railroad track, you look both ways because the track alone indicates the potential presence of trains. Even though none is visible at the moment, one can deduce that a train may have just passed or may arrive shortly at the crossing without having seen it. On a dirt road, tire tracks indicate the presence of automobiles, and to a hunter, game is indicated by tracks in earth or snow. Thus, you are familiar with the fact that if we do not actually see an object, its presence in the vicinity can be deduced by tracks it leaves behind. Cosmic rays, gamma rays, and certain radioactive particles also leave tracks in a specially prepared environment.

The environment to be used for this Inquiry is created in a cloud chamber (Figure 5). A cloud chamber is an enclosed space filled with a supersaturated gas that condenses and forms a vapor trail whenever an electrically charged particle (an ion) passes through it. The ions become the centers for the condensation of the supersaturated gas which then appears like streaks of fog in the cloud chamber. What will be observed in the cloud chamber is not actually a particle or ray itself but the track it has taken through the cloud chamber.

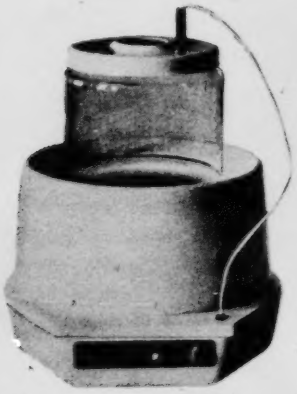


**Figure 5. A commercially available cloud chamber used for the detection of radioactive particles.** ***Photograph courtesy of Sargent-Welch Scientific Company.***

In this Inquiry you are to observe carefully radiation tracks from various sources and try to classify or identify some of their characteristics. This will not be an easy task, but careful observation of each event while looking for unifying or reoccurring patterns should allow you to classify several different kinds of tracks and record their characteristics.

MATERIAL (per group)

Aluminum foil (less than a square centimeter)
Cloud chamber and its associated materials, such as methyl alcohol, black dye, dry ice (8″ x 8″ x 1″ slab)
Paper (less than one centimeter square)
Radioactive emitters marked as unknowns 1 and 2
Ruler (millimeter, transparent)

PROCEDURE

*Radiation Tracks*

1. Review the construction and operation of the cloud chamber as directed by your teacher.
2. Set up the cloud chamber and place one of the unknown radiation sources in it as directed.
3. After conditions inside the chamber have stabilized, carefully observe the tracks of ionizing radiation.
4. Describe the types of tracks that tend to reoccur frequently.
5. After you have identified several reoccurring patterns, count and record in columns the frequency of each pattern observed over a given period of time.
6. Use a transparent ruler to estimate the length in centimeters of each type of path. How do they compare?
7. Summarize your observations indicating the number of different types of paths observed, their characteristics, and their frequency.
8. How do you account for the different patterns noted?

*Particle Energy*

Each of the tracks was produced by an ion that possessed a certain amount of energy. Energy in these cases is measured in terms of electron volts. Some indication of particle energy in million electron volts (Mev) can be gained by the length of the track. Compare the length of the tracks you measured with the following data and record the approximate energy in Mev for the tracks noted.

| *Track Length* | *Mev* |
|---|---|
| 1 cm | 2 |
| 1.6 cm | 3 |
| 2.5 cm | 4 |
| 3.5 cm | 5 |
| 4.6 cm | 6 |
| 6 cm | 7 |

*Penetration*

Earlier in this Block it was noted that various wavelengths of the electromagnetic spectrum have different penetrating powers. Does this difference in penetration also apply to alpha or beta particles or gamma rays?

For an explanation of these charged particles, refer to Appendix II ("What is Radiation?").

If the construction of the cloud chamber so allows, penetrating properties can be determined by placing a piece of paper or aluminum foil over the radiation source and observing if tracks are seen in the area beyond the shielded source.

With the radiation source shielded, repeat the observations in procedures 4 and 5 above. What types of tracks, if any, seem to be stopped by paper? What types seem to be unaffected by covering the radiation source? Repeat step 6. Does the shielding have an effect on track length? Prepare a statement regarding the penetrating ability of the particles creating the types of tracks noted above.

*Analysis of Data*

How many different types of tracks were noted in the cloud chamber? What is the significance of the length of the tracks? Which type is most numerous? Compare the data of students using radioactive emitter No. 1 with the data of those who used No. 2 when class data are pooled. What does this tell you about the radioactive sources used? Were certain types of tracks present in both samples? What is the significance of this observation?

Which type of path seems to have the greatest penetrating ability? What does this tell you about protection from radiation?

*Problems*

Remove all radioactive sources from the cloud chamber. Observe the chamber and record your observations. What explanation can you give for these observations? Design an investigation to test your hypothesis as to the source of this radiation.

Do particles passing through the cloud chamber have an electrical charge? How might this be checked simply? How could you determine whether the charge was positive or negative?

# INQUIRY IV *HOW CAN WE KNOW WHEN RADIOACTIVE MATERIALS ARE PRESENT?*

In the previous Inquiry, the ionization trails in the cloud chamber indicated the presence of radioactive particles and rays. However, the cloud chamber is not a convenient device with which to detect the presence of radioactivity. For one thing, it would be difficult, using a cloud chamber, to localize sites of radioactivity. In this Inquiry, you will learn how film can be used to detect the presence of particles and how to identify the sites from which they came. During the course of this Inquiry be alert for applications of this technique, known as autoradiography, to the study of biological systems.

MATERIAL (per group)

Cartridge pen with empty cartridge (2 of different colors or distinctive markings)
Cardboard [2 pieces, 13 x 18 cm (5″ x 7″)]
Film [No Screen Medical x-ray, 13 x 18 cm (5″ x 7″) or Kodak Royal Pan 10 x 15.5 cm (4″ x 6″)]
Film developer, 1 liter
Film stop bath, 1 liter
Film fixative, 1 liter
Film holder, or black envelope, or black paper (30 x 40 cm)
Forceps
Gloves, plastic, disposable
Laboratory coat
Needles, hypodermic, No. 26 (2)
Nonradioactive sodium phosphate solution, 2 ml
Notebook and paper
Paper, 7 x 9 cm (2¾″ x 3½″)
32-Phosphorus solution (radioactively labeled sodium phosphate), 1 microcurie per 2 ml, 2 ml
Radiation warning sign
Radiation warning stickers
Radiation warning tape
Rubber bands
Scissors
Stop bath, 1 liter
Syringe, hypodermic, 1 ml (2)
Trays, developing (3)

PROCEDURE

1. With the hypodermic syringe and needle, fill one clean and empty fountain pen cartridge with the $^{32}P$ solution. Place the filled cartridge in the pen. Mark carefully the pen containing the $^{32}P$.
2. With the second syringe and needle fill the second cartridge with the nonradioactive sodium phosphate solution and place the cartridge in the second pen.
3. On a piece of 7 x 9 cm notebook paper that has been divided in half by a penciled line, write the symbol $^{32}P$ or your initials, as you prefer, on the right side of the paper, using the $^{32}P$ solution. Each member of your team can write on the same side of the piece of paper if desired.
4. Repeat the procedure in 3 above using the second pen filled with the nonradioactive sodium phosphate, writing this time on the left half of the paper.
5. Thoroughly dry the paper.
6. In a darkroom, using no more than a photographic safe light, unwrap a piece of x-ray film. Hold it emulsion side up. (You can cut off the upper right-hand corner to identify the emulsion side.) Do not touch the film surface with your fingers. The film must not be exposed to any light until after it is developed. Place the dry notebook paper against the film. Either place both in a film holder or between 2 pieces of cardboard. Hold this cardboard sandwich together with rubber bands and then place it either in a black envelope or wrap it in black paper and seal it with rubber bands. Identify this package with your name or group number. Do not stack the films of other students on top of or beneath yours. The radioactivity from their paper may also affect your film.

After 24 hours have elapsed, open the film package or holder in a darkroom and separate the film from the paper. Put the paper and cardboard in the container marked "Radioactive Waste."

7. Develop the film as follows:
   a. Place the three developing trays in a row on a surface in the darkroom. Into the tray on the left, pour sufficient developer to completely cover the film. Pour the same amount of stop bath into the tray in the middle and a similar amount of fixative into the tray on the right. Do not contaminate one solution with the other. Do not spill or splash solutions. *Cau-*

*tion*: Photographic chemicals stain and are harmful to clothing. Make sure that no fixative gets into the developer.

b. Place the film in the developer, covering it completely, for about 4 minutes at 68°F. Occasionally wiggle the film in the developer with forceps.
c. After 4 minutes, remove the film from the developer tray with forceps. Let the excess developer drip back into the tray and then transfer the film to the stop bath for about 15 seconds. Remove with rinsed forceps and allow to drain. Transfer to the fixative for 5 minutes or until clear.
d. Rinse the film in cool, running water for 30 minutes. Hang the film up to dry.

### Analysis of Data

What could you see on the dried notebook paper on which you had written? Compare the right and left halves of the developed photographic film. How does the nonradioactive phosphorus solution affect the film? How has the $^{32}P$ solution affected the film? How can you account for these differences? To what uses might photographic film be put to detect radiation? What other instances do you know in which film is affected by radiation we cannot see? How could the film be used to detect the amount of radiation? Would the film be a good or a poor detector of amount of radiation? Why or why not?

### Problems

Design experiments to determine the effect of radiation on film by varying the times of exposure or distances from the source to the film.

In many areas where radionuclides are used, workers wear film badges. These are simply little carriers into which a piece of unexposed film is inserted. The film is removed and developed at stated intervals. What would the state of the film indicate about the environment of the worker? Design and, if time allows, perform investigations to determine the types of radiation to which the film is sensitive.

# INQUIRY V *HOW IS THE GEIGER-MULLER COUNTER AFFECTED BY RADIATION PRESENT IN THE ENVIRONMENT?*

A review of Appendix IV on how to operate a G-M counter shows that it is sensitive to radiation but not equally sensitive to all types of radiation. You also know from earlier Inquiries that radiation is naturally present in the atmosphere. What is the relationship between this naturally occurring radiation and the operation of the G-M counter?

You perhaps noticed in the work with the Geiger-Muller counter that even with no source nearby, the counter was registering radiation events. This activity recorded without any specific radioactive source in the vicinity is referred to as the background count. We know that the recorded counts are in response to radiation. But where does the ionizing radiation come from with no specific radioactive source in the immediate vicinity? This inquiry will concern itself with the observation of such background counts and hypotheses as to their source.

MATERIAL (per group)

Foil, aluminum, 12″ x 12″ piece
Forceps
Geiger-Muller counter with holder or ring stand and clamp
Lead and/or iron sheets, approximately 5″ x 5″ (6)

PROCEDURE

1. Hold the Geiger-Muller tube vertically with the window end down and the cover closed. Mount in either a holder or by means of a clamp to the ring stand.
2. Check the high voltage of the counter to insure that it is set to the proper, previously determined operating voltage. (Check Appendix IV for detailed instructions.)
3. Be sure that no radioactive materials are near the tube.
4. Take counts during periods of from 5 to 10 minutes. Record the results.
5. Record the count with the tube window uncovered.
6. With the shield again covering the window, wrap the tube in several layers of aluminum foil. Again record the count.
7. What does the data obtained with the shield covering the window and the aluminum foil around the tube tell you about the type of radiation producing the background count?
8. On the basis of your current knowledge of radiation and with reference to the introductory materials in this Block, list the possible sources that could account for this background radiation. Your list will form a basis for further discussion of this Inquiry.

FURTHER PROCEDURE

Place a piece of iron or lead flat on the laboratory table. Place similar pieces in such a way as to form a four-sided box open at the top. Place the Geiger-Muller tube carefully inside the box and cover the box, making sure that the lead cable is not crushed by providing a small opening for it. Record the effect of this thick metal box on the background count. What is the thickness of the iron or lead being used? Based on the reduction in counts per minute and the thickness of the metal, by what percentage is background radiation reduced with a given thickness of metal shielding?

ANALYSIS OF DATA

What seems to be the average background radiation level in your classroom? By what percentage is this reduced by having the tube window covered? By having the tube wrapped in aluminum foil? By placing the tube in a metal box? What do you think would be required to shield the background radiation to zero?

Because background radiation is always present, it influences all counting done with the Geiger-Muller counter. Therefore, for an accurate count, background radiation must be subtracted from the total count to give you the net activity of the sample being tested. A simple formula for this determination is $R_t$ (total count) $- R_b$ (background count) $=$ net activity of the sample.

Would you assume that background radiation is constant? Why or why not? If it is not constant, what factors must be taken into consideration during any experiment that will extend over a prolonged period of time? What is the biological significance of background radiation? Prepare a summary statement that answers the question in the title of this Inquiry.

## Problems

Experiment with different materials to determine their effect on reducing background radiation. Does the time of day have anything to do with the intensity of background radiation? Would you expect it to be the same at night as in the daytime? Test various areas in your immediate locality for background radiation. How does it vary, if at all? How would you account for this?

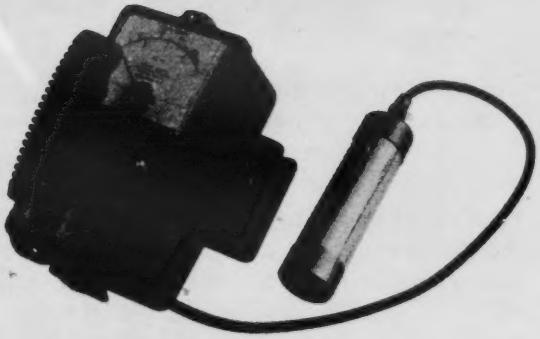


Figure 6a. A portable battery-powered counter. The end window of the Geiger-Muller tube is protected by a removable cap-like shield. Counts per minute are recorded on a survey meter and also can be heard as a series of clicks.

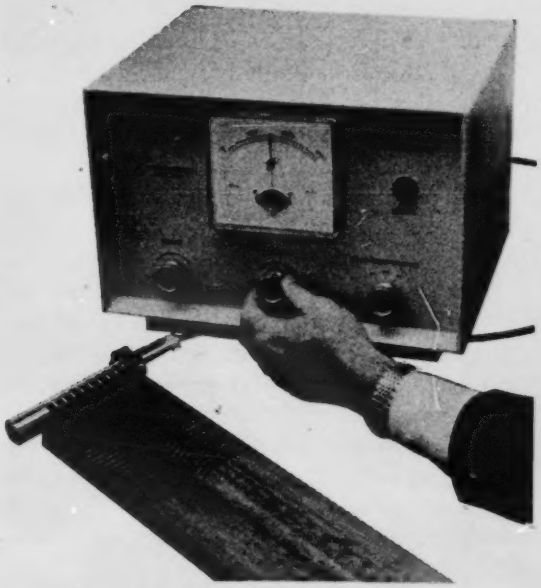


Figure 6b. This counter uses 115-volt alternating current and is equipped with a side-window Geiger-Muller tube. The calibrated board on which the tube is temporarily mounted can be used in both a vertical and a horizontal position.

# INQUIRY VI *WHAT DOES A GEIGER-MULLER COUNTER DETECT?*

The first response to the question in the title is probably, "Counts per minute." But counts of what? Remember the work with the cloud chamber? To what kinds of radiation was it particularly sensitive? Was the photographic film used for the autoradiograms equally sensitive to radiation of various types? On the basis of these data from previous Inquiries, what hypotheses can you state regarding the anticipated sensitivity of the Geiger-Muller tube to various kinds of radiation? Data relative to your hypothesis can be obtained by a series of experiments designed in a similar fashion to those you performed with the cloud chamber.

You will be provided with three unknown radiation sources. They will be numbered 1, 2, and 3. You will be allowed to use paper, aluminum, and lead as filters. From the reaction of the tube in response to your experiments, you are to deduce the major type of radiation from each of the three unknowns.

MATERIAL (per group)

Filters, paper, aluminum, lead
Forceps
Geiger-Muller counter with clamp ring and stand (holder optional)
Radioactive samples, 3 unknowns
Ruler, 10 cm

PROCEDURE

1. Be sure you are familiar with the operation of the Geiger-Muller counter. Review, if necessary, Appendix IV ("How Do You Operate a Geiger-Muller Counter?")
2. Mount the Geiger-Muller tube in a vertical position, window down (shield closed) in either a holder or a clamp mounted on a ring stand. Be sure the scaler voltage is properly set as explained in Appendix IV.
3. Place one of the numbered samples either at the bottom of the card mount holder or on a piece of glass mounted on a ring fastened to a ring stand no more than 10 cm below the Geiger-Muller tube.
4. Record the counts per minute registered on the scaler or rate meter. If no counts above background are recorded, place the sample on the next highest shelf in the holder or raise the ring stand, recording in each case the distance from the sample to the shield of the tube.
5. Continue raising the sample and recording counts at each distance.
6. Again lower the sample to the bottom of the ring stand or holder as in 3 above. Remove the shield from the window and repeat procedures 4 and 5, recording counts per minute at each level.
7. Place the sample at a point where the count no longer increases rapidly when the sample is brought closer to the tube, but in no case closer than 1 cm to the tube.
8. Now, carefully place a paper filter over the radioactive sample. Then replace it with an aluminum foil filter, and finally replace this with a lead filter. Record the count for each filter.
9. Repeat steps 3-8 with the other two radioactive samples.

ANALYSIS OF DATA

What was the effect of distance on the counts per minute? Compare the three samples. Which gave the greatest number of counts per minute? Which the least? Did any sample not register on the counter? What was the effect of use of the paper filter? The aluminum filter? The lead filter? From the data of your earlier Inquiries, which type of radiation could be shielded by paper? By aluminum? By lead? On this basis, what type of emitter do you deduce sample 1 to be? Sample 2? Sample 3?

With the identical counting geometry (see Appendix IV), to which sample was the Geiger-Muller counter most sensitive? The least sensitive? How do you account for this? What assumptions have to be made in order to make the statement that the counter is more sensitive to one kind of radiation than to another? For the detection of what type of radiation would the Geiger-Muller counter be most effective?

After all of your data have been recorded, have they supported or refuted your original hypothesis regarding the sensitivity of the Geiger-Muller tube to various kinds of radiation?

Your teacher will tell you the nature of the source material in samples 1, 2, and 3. If your data do not agree with the sources as revealed,

re-examine your techniques for possible explanations. How do these data relate to data of the previous Inquiry? Write a summary statement of the significance of these data for biology.

### Problems

Using the above techniques you can determine the major types of radiation emitted from other unknown samples that may be available. Try different materials as filters to measure their effects.

You noticed that the counts per minute varied with the distance of the sample from the end of the Geiger-Muller tube. Design investigations to show the influence of distance on radiation.

How effective are biological materials as radiation filters? Make filters of scales, feathers, fur, frog skin, or other biological materials and test them. Compare the results. How effective is a leaf as a radiation filter? Would an aphid under a leaf receive as much radiation as one living on top of a leaf? Design investigations to answer these and similar questions that may arise in your discussions.

# INQUIRY VII *HOW MUCH RADIOACTIVE MATERIAL FALLS TO EARTH?*

The term "fallout" is commonly used in this nuclear age. Fallout is made up of airborne particles containing radioactive materials that settle to earth (Figure 7). There has always been some fallout from natural causes. Within the last decades, as man has set off nuclear devices, the amount of radioactive material in the atmosphere has increased. So has the amount of fallout. In the case of short-lived radionuclides, this is not a serious problem. But, with ones such as 90-strontium, with a half-life of 28 years, and 14-carbon, with a half-life of 5730 years, there are possible hazards. Not only are these radionuclides long-lasting, but both are taken up by plants and animals. 90-strontium, for example, replaces calcium in bone, and the potential danger from its radioactivity increases markedly when it becomes an emitter from within the body.

You have established a background count for your immediate area. Is this background count evidence of fallout? In a way it is. But if we concentrate some kind of material that has had the opportunity to pick up radioactive particles from the atmosphere, we should be able to measure a count higher than that of background. This difference we consider a measure of fallout, even though the total background also must be considered of this nature.

What materials are available that could pick up radioactive debris from the atmosphere? You might think of rain, snow, and dust, among others. What other kinds of substances could you suggest that might be sources of fallout?

MATERIAL (per group)
Aluminum foil, 4″ x 2″
Geiger-Muller counter with holder or ring stand and clamp
Pen, ball-point
Plastic wrap, 3″ x 3″
Rubber band, large
Sack, cheesecloth or other fabric, 4″ x 4″
Scissors
Tape, cellophane
Vacuum cleaner

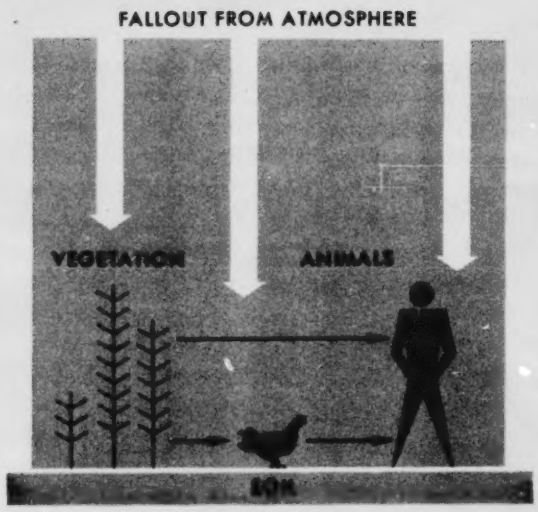



Figure 7. Atmospheric fallout can be taken up by both plants and animals and passed from one to another in the food chain.

PROCEDURE

1. Fasten the cheesecloth sack or one of some other fabric over the intake hose of a vacuum cleaner by means of a large rubber band.
2. Mark a circle on the sack around the edge of the opening in the hose with a ball-point pen.
3. Stick the hose out of the window and secure it firmly. Turn on the vacuum cleaner and allow it to run for 15 minutes.
4. Fold the piece of aluminum foil in half to form a 2-inch square.
5. Turn off the vacuum cleaner and carefully remove the sack. Cut out the circle previously marked. Center it on the piece of aluminum foil.
6. Place the aluminum foil with the circle of cloth beneath the end-window of a mounted Geiger-Muller tube. Open the shield and count for at least 5 minutes. Record your results.
7. Place the piece of plastic wrap over the top of the circle of cloth and fold it back under the aluminum foil. Fasten it in place with cellophane tape on the back of the aluminum. Repeat the counting technique in 6 above.
8. After from 30 minutes to an hour, take another count of the plastic-coated sample. Record your data.

### Analysis of Data

Compare the counts per minute between the plastic-wrapped sample and the uncovered one. Subtract the background count as determined in Inquiry V from your figures. If we consider the difference to be because of fallout, what statement can you make regarding the status of fallout at the time this sample was taken? Do you regard this amount of fallout as significant? Why or why not? How do the counts taken a half hour later compare with the initial ones? How do you account for this difference, if any? What is the biological implication of these data?

### Projects

What type of radiation is this fallout? Use filters as a possible way of determining this. Your teacher will suggest other possible sources to be examined for fallout. Periodic fallout checks over a span of time can give comparative information about variation in amount of fallout. Investigate the phenomena of half-life and decay of radionuclides in relation to fallout. Check Appendix II for a discussion of these terms. Determine which radionuclides are taken up by organisms. What hazards are involved from this uptake?

# INQUIRY VIII *HOW DO PLANTS REACT TO RADIONUCLIDES?*

In Inquiry I we calculated the LD-50 (lethal dose–50 percent) for ultraviolet light on a yeast population. From this we learned of the lethal nature of radiation. But is all radiation lethal? If radioactive materials are in an organism, do they produce the same effects as if the organism were subjected to an equal amount of external radiation? What would be the effect of uptake of a radionuclide by a plant? Would you expect the plant to be killed or damaged? Inquiry I was concerned with a microorganism, but how does a multicellular organism react? Are each of its cells as sensitive as individual microorganisms? What happens to a radionuclide incorporated into a multicellular plant, and how does the plant react? Answers to only some of these questions can be provided by a simple inquiry. The others may serve as a basis for further future study.

Some idea of the metabolic requirements of a multicellular organism and the path taken by a specific element can be obtained by the use of radionuclides. For this purpose we will use an element which is normally taken up by plants for their metabolism. A common element required by plants is phosphorus and the usual form of administering it is in phosphate.

To determine the effect of a radionuclide on a multicellular plant we will use the phosphorus in a radioactive sodium phosphate solution and a tomato plant. With your knowledge of plant structure and function, where would be the most reasonable site to apply the phosphate solution? Would other places on the plant serve as well?

MATERIAL (per group)

Cardboard, 13 x 18 cm (5″ x 7″), (2, or 4 if plants are large)
Envelope, black, for 13 x 18 cm cardboards (or black paper or film holder)
Film developer, 1 liter
Film stop bath, 1 liter
Film fixative, 1 liter
Film, no screen x-ray, 13 x 18 cm (5″ x 7″)
Forceps
Needle, hypodermic, No. 26
32-Phosphorus (labeled sodium phosphate $NaH_2{}^{32}PO_4$), 0.3 microcurie
Pipette, medicine dropper, with drawn-out tip
Plastic wrap, 1 foot
Rubber bands
Sticks, ice cream or equivalent (4″ to 8″ in length for propping plant leaf and supporting plant)
Stop bath, 1 liter
Syringe, hypodermic, 1 ml
Tape, cellophane
Test tubes, 30 ml (2)
Test tube holder
Tomato or bean seedlings (2), 4″ to 8″ in height
Trays, developing (3)
Water, 15 ml

PROCEDURE

1. Search for a horizontal leaf on one of the planted seedlings. If none is found, prop a leaf in a horizontal position by means of sticks.
2. Using the medicine dropper pipette, place a small drop of $^{32}P$ solution in the center of the leaf. Be careful that none of the solution runs off. Allow the liquid to dry before moving the plant.
3. Write a statement in your notebook as to what you believe will be the result of having applied $^{32}P$ to the leaf.
4. Carefully remove a second plant from the vermiculite or soil in which it is growing. Loosen the vermiculite or soil with a stick or pencil so that the roots can be pulled out without damaging them. Carefully shake off as much of the vermiculite from the roots as you can.
5. Place the root system of this second plant in a 30 ml test tube. Add 15 ml of water. Support the plant on a stick taped to the side of the test tube and place the tube in a holder. Carefully add 0.3 microcurie of $^{32}P$ in 1 ml of sodium phosphate solution to the test tube using the hypodermic syringe. Shake the test tube carefully to mix the $^{32}P$ with water. Be sure the roots are covered by the solution.
6. Place this plant beside the other one and allow both to stand for 24 hours. Why should the plants be kept together?
7. At this point analyze the experimental design for defects. What has not been done so far that should be done for every experiment? Decide as a class how to rectify this omission

before proceeding further.

8. After 24 hours remove the one plant from the soil or vermiculite and the other from the test tube. In the sink rinse the $^{32}P$ solution from the roots. Be careful not to splatter. Remember, do not touch the plants with your hands—use forceps or gloves. Ask your teacher whether to save or to dispose of the $^{32}P$ solution remaining in the test tube.

9. Using your forceps, place the plants on the 13 x 18 cm piece of cardboard. Spread out the leaves so that they are flat but not lying on top of one another. Spread out the roots as much as possible. Bend the stems if necessary but keep all parts of the plant on the cardboard. If both plants are too large to fit together on one cardboard they may be placed on the separate pieces of cardboard and steps 10 through 13 repeated for each plant. Identify each plant (whether from test tube or soil) by labeling it on the cardboard or by drawing in your notebook.

10. Cover the cardboard and plants completely with a piece of plastic wrap. Make sure that the plants cannot move on the cardboard. Use the cellophane tape to secure the plastic wrap on the side of the cardboard opposite the plants.

11. In a photographic darkroom place a piece of no screen x-ray film, emulsion side down, over the plants on the cardboard and cover it with another piece of cardboard. Fasten this sandwich together with two rubber bands and place it inside a black envelope.

12. Up until this point all the procedures for the class have been the same. At this time a third of the group should store the film in the dark for one day, another third for two days, and the remaining third for three days.

13. At the proper time open the film package in the darkroom. Discard the cardboard, plastic wrap, and plants in the radioactive waste container. Develop the film as outlined in Inquiry IV.

### Analysis of Data

On the basis of the autoradiogram what statement can be made about the relative absorption of $^{32}P$ in each of the two experimental plants? Note the distribution of $^{32}P$ in the plant. What does this distribution tell you about the localization of phosphorus in the growing plant? Think of some of the common metabolic processes in which phosphorus may take part. Do these data indicate to you some reason for the distribution of phosphorus? What radiation effects, if any, were noticeable in the plants after 24 hours? What do you suppose would be the effect of $^{32}P$ on the plant after a longer period of time? How can you check your answer to this question?

How does the phosphorus move through the plant when deposited on a leaf? How does this path differ from that of the phosphorus absorbed by the root? If phosphorus can enter the plant through the leaf would spraying foliage seem an effective way of fertilizing a plant?

Do you have any indication that phosphorus is used more in one part of the plant than in another? What indications, if any, do you have that phosphorus is localized or stored in any particular part of the plant? In what tissues, if any, does it seem to be localized? Answer as many of the questions posed in the introductory paragraph of this Inquiry as you can on the basis of your experimental data.

### Problems

If a tomato plant was used in this Inquiry, would you expect similar responses to phosphorus if other types of plants had been used? Design an investigation to test your hypothesis.

Design an investigation to determine the effects of $^{32}P$ on the plant over a long period of time. Can phosphorus be absorbed through plant parts other than leaves and roots?

What relation does the movement of phosphorus bear to the process of photosynthesis? By repeating this experiment with plants in the light and in the dark, data on your hypothesis may be obtained.

# INQUIRY IX *WHAT HAPPENS TO A RADIONUCLIDE INJECTED INTO ANIMALS?*

In the previous Inquiry you saw how phosphorus was distributed in a plant. If 32-phosphorus is injected into an animal, what are the results? On the basis of your knowledge of animal physiology formulate hypotheses as to what you believe will happen. In this Inquiry we will use radioactive phosphorus injected into a frog. The data from this Inquiry should support or refute your hypotheses.

MATERIAL (per group)
Cardboard, 13 x 18 cm (5″ x 7″) (2)
Chloroform, 5 ml
Container for frog
Cotton, absorbent, small pad
Dissecting kit
Dissecting pan with wax bottom
Envelope, black, for 13 x 18 cm cardboards (or black paper or film holder)
Film developer, 1 liter
Film stop bath, 1 liter
Film fixative, 1 liter
Film, no screen x-ray, 13 x 18 cm (5″ x 7″)
Frog
Jar with lid, 8 oz. or larger for anesthetizing frog
Needle, hypodermic, No. 26
Pins (8)
Plastic wrap (8″ x 10″), (2)
32-Phosphorus solution (labeled sodium phosphate $NaH_2{}^{32}PO_4$), 1.0 microcurie
Rubber bands
Scissors
Sodium phosphate solution, nonradioactive
Syringe, hypodermic, 1 ml
Tape, cellophane
Trays, developing (3)

PROCEDURE

1. Inject a frog with approximately 1 microcurie of $^{32}P$ in 1 ml of sodium phosphate solution. This is done as follows:
   a. Hold the frog firmly. Insert the hypodermic needle through the thigh muscle. Direct the needle forward to the dorsal lymph sac. (The space under the loose skin of the lower middle back of the frog.) Inject the sodium phosphate solution into the space under the skin. Be careful not to allow the needle to extend through the skin or into your fingers. When the needle is withdrawn the muscle seals the injection site and prevents the loss of the $^{32}P$ solution.
   b. Place the frog in a marked container where it will have both water and air, but will not be able to splash water outside the container. Leave the frog in the container for from 24 to 48 hours before proceeding with step 2.
2. Kill the frog by overanesthetizing with chloroform. Pour about 5 ml of chloroform onto a cotton pad and place in a large jar. Put the frog inside and cover the jar. Wait about 10 minutes until you are sure the frog is dead.
3. Place the frog, ventral side up, in a wax-bottom dissecting pan and pin the legs in an extended position.
4. Carefully open the body cavity as directed. Remove the liver and spleen and place them side by side on a piece of plastic wrap. Also remove the heart and one lung and place them on the plastic wrap. Carefully slit the skin of the left hind leg from the ankle to the pelvic region. Cut the muscle away from the bone and place a piece of muscle on the plastic wrap.

   At the pelvic joint of the left hind leg separate the upper end of the thigh bone from the pelvic girdle. Be careful not to break the bones. Two bones are attached to the thigh bone. Separate their lower ends from the bones at the end of the leg. You now have a pair of bones attached to each other and to the thigh bone by ligaments. Most of the muscle has been removed earlier. If any remains remove it now. Place these bones on the plastic wrap with the other parts.
5. It is considerably more difficult to dissect but, if time allows, remove the brain and place it with the other parts.
6. Arrange the parts on the plastic wrap so that they will fit within a 13 x 18 cm rectangle. Cover the parts with a second sheet of plastic wrap and press it down gently so the frog parts and the two pieces of plastic wrap stick together as a unit.
7. On one of the 13 x 18 cm pieces of cardboard trace the outlines of the parts placed on the plastic wrap and label them. You will need

this diagram later to identify the position of the parts on the film.

8. Place the plastic-wrapped parts against the cardboard so that each matches the labeled diagram. Fold the excess plastic wrap around the edge of the cardboard and firmly secure it to the back of the cardboard with cellophane tape.

9. In a darkroom place a sheet of x-ray film over the parts and cover it with the second 13 x 18 cm piece of cardboard. Fasten the cardboards, the film, and the plastic-wrapped parts together with rubber bands and place the packet in a film holder or black envelope.

10. Allow the parts and the x-ray film to remain in contact in the dark for three days.

11. After three days remove the film in a darkroom, discard the plastic, the frog parts, cardboards, and the rubber bands in the radioactive waste can. Develop the film as outlined in Inquiry IV.

### Analysis of Data

Examine the autoradiogram of each of the frog parts. How do the various parts selected compare as to radioactivity? On the basis of your answer to this question list the parts in order of the amount of $^{32}P$ they apparently contain from most to least. On the basis of this data what kind of hypothesis can you formulate regarding phosphorus metabolism in the various parts concerned?

If the time interval between the injection and the examination of the parts were two weeks, what would you expect the results to be? How can you account for this answer? With what you have just observed how can you be sure that the results you have seen are due to the radioactive phosphorus you injected? Why could they not be due to natural radioactivity within frogs? How could you rule out this possibility? What would you expect the effect of the radioactive phosphorus to be on the frog? How can you gather data to test this hypothesis?

Compare the frog tissues with those of the plants in the previous Inquiry. Contrast the distribution of radioactive phosphorus in both organisms.

### Problems

Frogs may be injected with other radionuclides such as 131-iodine or 45-calcium. Autoradiograms could show specificity of these elements for various organs.

Various time intervals can be used to trace the movements of radionuclides in the organs concerned and their rate of elimination, with allowances made for decay.

Animals other than frogs can be used—mice and fish, for example. Radionuclides need not be injected. They can be administered in drinking water or added to the water of a small aquarium to determine absorption by an organism such as a fish.

It is interesting to follow the path of an element through stages of a life cycle of an organism. This is a long-term project and a radionuclide with a long half-life is necessary. It might first be introduced into a plant known to be the food of a caterpillar. A number of caterpillars might then feed upon the plant. An examination of the tissues of sacrificed caterpillars, pupae, and adult insects would provide interesting data on localization and utilization of elements during the life cycle of an insect.

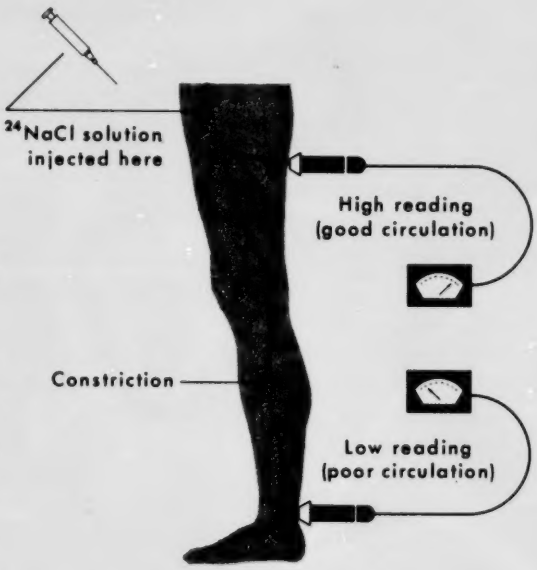



Figure 8. Radionuclides are used as tracers in a wide variety of ways. Not only can they be localized in specific organs as in this Inquiry, but they can be used to detect rates of flow. In this case, labeled sodium is used to detect the site of restriction of blood flow to the lower leg.

# INQUIRY X *WHAT EFFECT DOES IRRADIATION HAVE ON SEEDS?*

In the first Inquiry we observed that radiation can inactivate and kill organisms. Subsequent Inquiries have been concerned with detection and uses of radiation. Let us now return to radiation effects. From what you now know, it should be fairly easy to devise investigations to determine the effects of radiation on a wide variety of organisms. It should only be necessary to irradiate the organism concerned with given dosages of particular radiation and observe the results. This type of experimental design is relatively simple; it is in the observations and their interpretations that difficulties arise. First, one needs to know the normal sequence of events with which the data obtained from an irradiated specimen can be compared. What does this then mean in terms of experimental design?

As an example of such an irradiation study, we will use seedling growth. Seeds can be purchased already irradiated with various dosages of radiation from 10,000 to 40,000 roentgens. They can be irradiated locally if a high-energy source is available. Even with such a simple experimental design, requiring only irradiated seeds and a control, there is a wide range of possible variables. For example, this design can be used to determine the effects of various irradiation dosages on seeds of similar types. It can be used to compare irradiation effects on seeds from various plants. It can be used to check the effect of irradiation on germination time, on the first appearance of leaves or roots, or on growth plotted against time. What other variables can be tested by this design?

## PROCEDURE

1. You are to select a variable you wish to study. Write your hypothesis concerning what you believe to be the effect of irradiation upon the variable you have selected.

2. Write out the experimental design that will provide the data necessary to evaluate your hypothesis. Included in the design should be the type of seed you are using, the number of seeds, the amount of irradiation, the type of controls, the time you expect the experiment to take, and a complete list of materials.

3. After you have received your teacher's approval, assemble the required materials and begin the investigation. During the course of your observations you will want to note carefully whether the expected changes do indeed occur, as well as to observe those changes, if any, that occur that you had not anticipated.

4. As your experiments undoubtedly involve some measurements, how are you going to prepare the data for analysis? Will you place them in tabular form? Will you make a bar graph or a line graph? Be prepared to present your data in a form that can be understood by other members of the class.

5. After the data have been assembled, analyze the data and prepare a summary of your results. What has your experiment shown about the effects of radiation on seeds? What kinds of questions can you now answer on the basis of your experiment?

6. Be prepared to present the results of your experiment to the class and to discuss them.

## PROBLEMS

This basic experimental design leads to a variety of long-term projects. For example, the plants from the irradiated seeds can be grown to maturity and the effects of irradiation noted on flowers, fruits, seeds, etc. Experiments can be conducted to show relative effects of radiation at various stages in the plant's growth and development. Seeds from the plants grown from the irradiated seeds can be collected and, in turn, planted to observe any hereditary effects of irradiation. In this latter case, individual flowers must be covered by transparent bags to insure self-pollination.

Seeds can be soaked in solutions of radionuclides previously used in class, such as $^{32}P$, and the effects of this type of radiation contrasted with that of others.

# INQUIRY XI *HOW CAN YOU DETECT THE MOVEMENT OF MATERIALS THROUGH AN ECOSYSTEM?*

One of the uses to which radionuclides are put is to act as tracers (see Appendix III). In Inquiries VIII and IX you were able to trace the distribution of phosphorus in a plant and in an animal. Similarly, tracers can be used to follow an element through the principal components of an ecosystem. The basic experimental design is simple. It involves introducing a radionuclide at a given time in an ecosystem and, by detection instrumentation, measuring its uptake in various organisms. Different quantitative techniques can be used to measure uptake and concentration of the element concerned. For one illustration of such a technique, a simple aquarium will serve. To clarify the gathering of data and analysis of results, a single type of plant, such as elodea, and a single type of animal, such as the snail, are all that is required. The radionuclide will be introduced into the water and its uptake and accumulation in the plant and in the snail will be observed. A week or two prior to the time of initiating this Inquiry a balanced aquarium should be set up containing elodea and pond snails.

MATERIAL (per group)

Absorbent paper or paper towels, 3-cm-square pieces (20)
Aquarium, balanced (with elodea and snails)
Elodea plants
Forceps, extra long
Forceps, regular
Geiger-Muller counter with holder or ring stand and clamp
Graph paper, semilog, 5-cycle, 1 sheet per student
Light source
32-Phosphorus solution (0.5 to 1.0 microcurie per liter of aquarium water)
Pipette (2 ml or larger)
Planchets or bottle caps (30)
Rod, glass, stirring
Snails, pond (a minimum of a dozen per aquarium)
Wax paper or plastic wrap, 3-cm-square pieces (20)

PROCEDURE

1. Pipette a 2 ml sample of water from the balanced aquarium into a planchet or a bottle cap. Evaporate and measure its activity with an end-window Geiger-Muller tube. (Remember that all measurements should be taken the same distance from the tube.)

2. Remove a leaf of elodea from a plant growing in the balanced aquarium. Remove the excess water by placing it for about a minute on a small (3-cm-square) piece of absorbent paper or paper towel backed with a similar piece of wax paper. Place it in a bottle cap or planchet and measure its activity as in 1 above.

3. Repeat step 2 with a single snail. As the snail is not damaged, it can be returned to the aquarium after being checked for activity.

4. Add 0.5 to 1.0 microcurie of 32-phosphorus per liter of water to the balanced aquarium. Stir the water with a glass rod gently so as not to disturb the organisms.

5. Again determine the activity of the water, the snail, and the leaf as in steps 1-3. Record your data.

6. Discard the pieces of paper and bottle caps in the radioactive waste container.

7. Leave the aquarium in an area lit by moderate natural light (not direct sunlight) with necessary supplement by artificial light to provide a relatively constant level of illumination over a given time period daily (10 to 12 hours).

8. The following day and on alternate days thereafter for a period of 2 weeks repeat steps 1-3, recording the data and discarding the paper and caps as in 6 above.

9. Plot this data on a graph showing counts per minute on the vertical axis and time in days on the horizontal axis for the water, the snail, and the elodea leaf.

ANALYSIS OF DATA

What happens to the counts per minute in the water? Between what two time intervals is the change the greatest? What happens to the counts per minute in the elodea leaf? For how many days is the count the highest? Between what two days is there the greatest increase in count?

How does the graph for the counts per minute in the snail differ from that for water and the elodea leaf? By what day have the counts in the snail reached their maximum? How do you ac-

count for these data? What do they tell you about the movement of an element through an ecosystem? After 14.3 days, (two weeks) only 50 percent of the radioactive phosphorus is still present. But how is it distributed at that time? Is the radioactivity of the water now 50 percent of what it was initially? For the elodea? For the snail? On the graph that shows the counts per minute for elodea, snails, and water in relation to time, plot the decay rate of $^{32}P$. Where is the $^{32}P$ being concentrated? Is all of the $^{32}P$ found in snails due to the fact that snails ate the elodea? What would happen if a second order consumer (a fish that ate snails) were added to this ecosystem? After two weeks would you receive a greater dosage of $^{32}P$ per unit of mass if you drank the water from the aquarium or if you ate the elodea? What does this tell you about the relative hazards of consuming contaminated materials?

## Problems

More complicated food webs can be set up. The introduction of several kinds of plants, fish, *Daphnia*, or crustaceans can add to the complexity of the ecosystem and provide alternate pathways for the $^{32}P$.

Other radionuclides, such as 14-carbon, can be used rather than 32-phosphorus. They will give somewhat different results.

In areas near the ocean, marine aquaria can be set up using shellfish, echinoderms, anemones, etc.

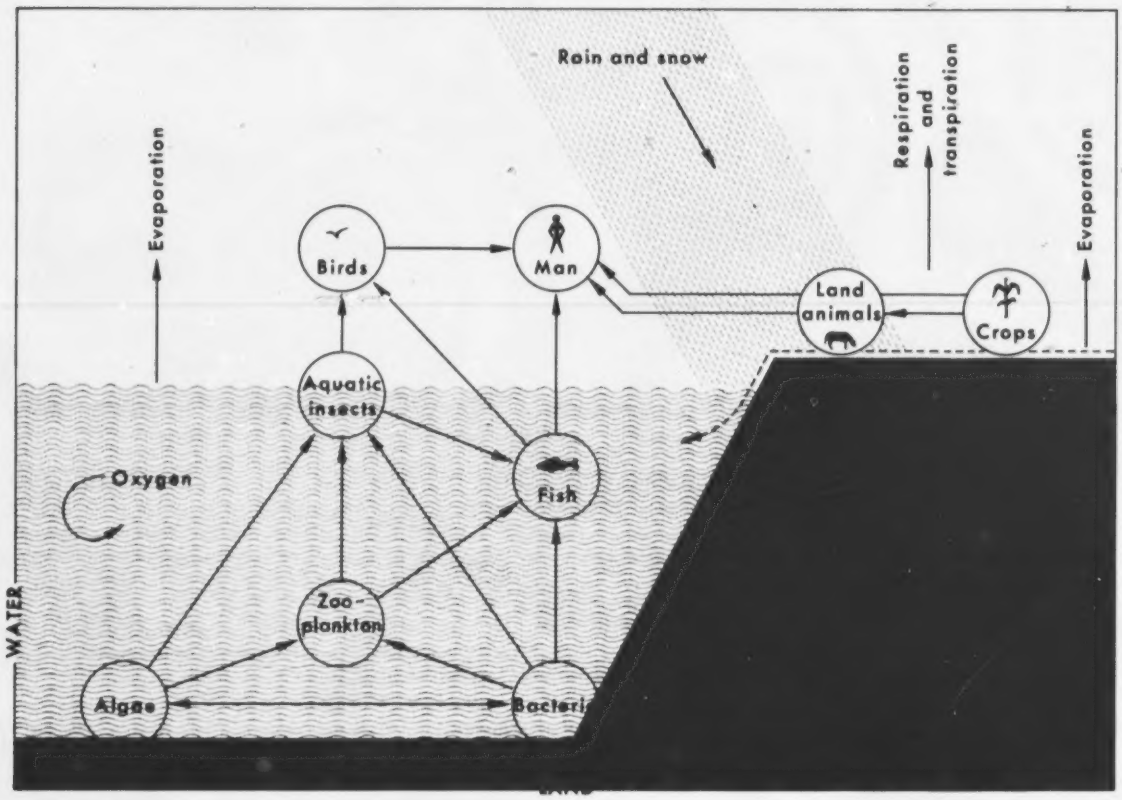



Figure 9. Diagramatic representation of the flow of radionuclides through a natural ecosystem. Contrast the complexities shown in this figure with the simple food chain used in this Inquiry.

# INQUIRY XII HOW CAN RADIONUCLIDES BE USED TO SOLVE BIOLOGICAL PROBLEMS?

In Inquiries VIII and IX, $^{32}P$ was used to show uptake of phosphorus in a plant and in an animal. These data told us something about the distribution of phosphorus in these organisms. From these data, certain hypotheses could be made about the role of phosphorus in plant and animal metabolism. These hypotheses then could be tested by further experimentation.

Are there more specific ways to use radionuclides in solving biological problems? For example, how might radionuclides be used to explain the events that occur during such a process as photosynthesis? We are all familiar with the simplified formula for photosynthesis whereby carbon dioxide and water, using light energy and chlorophyll, form carbohydrates and liberate oxygen:

$$6\ CO_2 + 6\ H_2O \xrightarrow[\text{chlorophyll}]{\text{sunlight}} C_6H_{12}O_6 + 6\ O_2$$

If you accept the above simple formula, you must assume that carbon dioxide and water are used in the presence of light to form the carbohydrate. If there is no light, there is no photosynthesis and no uptake of carbon dioxide or use of water for this purpose.

You are familiar with the fact that this summary omits many of the separate intermediate steps that occur. Many questions can be asked. For example, does the oxygen in the carbohydrate come from the water or the carbon dioxide? Or both? And what about the carbon itself? At what stage of the process is the carbon of carbon dioxide involved and what is its relation to light energy and chlorophyll? Could there even be other sources of carbon for the carbohydrates? Perhaps the carbon comes from elsewhere in the plant for the purposes of photosynthesis, and the carbon in the carbon dioxide is a replacement for that carbon so used.

On the basis of what you now know about radionuclides, you can probably think of ways to devise experiments that will show the role of the carbon in the carbon dioxide or the role of the oxygen in either water or carbon dioxide. Such experimental designs quickly become complex and require skills perhaps not yet mastered. However, you can use 14- carbon in $^{14}CO_2$ and, by varying the experimental techniques, demonstrate several features relative to photosynthesis. Using $^{14}C$ in the form of sodium carbonate ($Na_2{}^{14}CO_3$), you will compare the uptake of carbon dioxide (released from the $Na_2{}^{14}CO_3$ as $^{14}CO_2$) in the light and in the dark and attempt to localize it within the plant. Other questions regarding the role of $CO_2$ in photosynthesis may occur to you. An alteration of this basic experimental design can provide some data for answers.

MATERIAL (per group)

Acetic acid, 0.1*M* ($CH_3COOH$) 2 ml
Barium hydroxide or calcium hydroxide, 0.2*M*, 4 ml
50 ml beaker or small glass container (2)
Black paper or black paint to darken one of the two-gallon jars
14-Carbon in sodium carbonate ($Na_2{}^{14}CO_3$) aqueous solution, 20 microcuries
Coleus plant, potted, small (2)
Film developer, 1 liter
Film stop bath, 1 liter
Film fixative, 1 liter
Film holder or black envelope
Film, no screen x-ray, 13 x 18 cm (5″ x 7″)
Fumehood or properly ventilated area
Gallon glass jars, screwtop with lids (2)
Lamp as light source, 100 W
Needle, hypodermic, No. 26 (2)
Plastic wrap
Rubber bands
Syringe, hypodermic, 1 ml (2)
Tape, cellophane
Trays, developing (3)
Wire for beaker support in jar, 1-foot piece (2)

PROCEDURE

The procedure using each of the two, one-gallon jars is identical except for the fact that one jar must be light-tight. It can be covered with black paper or painted with flat, black paint. The following description for steps 1-4 will be given in terms of a single jar and plant, but the setups for both jars should be performed at the same time.

1. Place a small potted coleus plant in the bottom of the gallon glass jar.

2. Support a small beaker or similar glass container with wire within 4 cm of the top of the glass jar (see Figure 10). By means of the hypodermic syringe and needle, add 10 microcuries of $Na_2{}^{14}CO_3$ (sodium carbonate) in aqueous solution to the beaker. Cover the jar with plastic wrap, fastening it tightly around the jar mouth with rubber bands to form as tight a seal as possible.

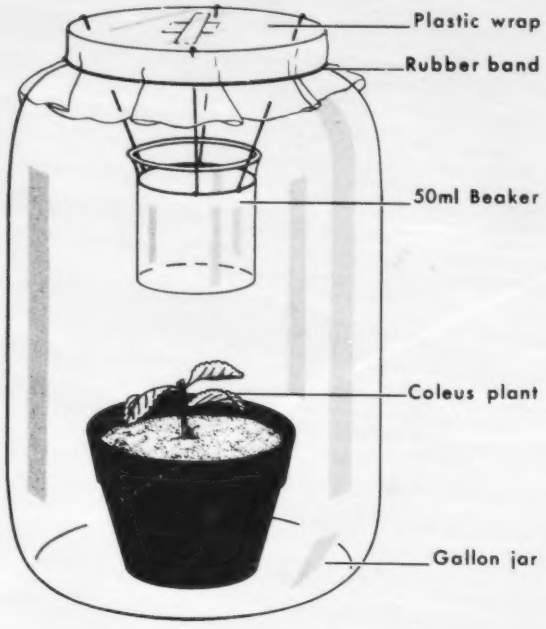



**Figure 10.** For Inquiry XII, two jars are prepared as shown. One, however, is light-tight. The beaker is suspended from the jar lip by means of wire and the plastic wrap forms a tight seal over both the jar and its contents.

3. On the plastic-wrap cover at a point directly over the sodium carbonate container, make a cross of cellophane tape about 3 inches long to reinforce the area.

4. Carefully draw 2 ml of 0.1*M* acetic acid into the second hypodermic syringe. Stick the hypodermic needle through the center of the crossed cellophane tape. Discharge the 2 ml of acetic acid into the sodium carbonate container. Seal the puncture hole with tape. The gas that is being generated is carbon dioxide, part of which is in the form of $^{14}CO_2$.

5. Place both jars carefully in a fumehood or in a well ventilated area. Illuminate the clear jar with a strong light. What is the purpose of the light?

6. Review the experimental design to this point. What is it designed to test? On the basis of your knowledge of photosynthesis from previous experiments in biology, hypothesize the condition of both plants when the experiment is done.

7. Depending on the time available, you may proceed with the experiment after 10 minutes, or you may wait until the next class period 24 hours later. Before opening the containers, the $^{14}CO_2$ must be removed. This may be done by introducing by means of the hypodermic syringe and needle either barium hydroxide or calcium hydroxide solution into the container of sodium carbonate at least an hour before the containers are opened.

If adequate ventilation is provided, the plastic seal can be cut with a knife and the container left in the closed chemical hood for at least an hour before examination.

8. Carefully remove the wire support and beaker and take the coleus plant from the jar. Place the plastic wrap, the beaker and its contents, the wire supports, and the rubber bands in the jar and seal with the lid until step 11.

9. Remove 1 or 2 leaves from the plant kept in the light and prepare an autoradiogram following the procedures outlined in Inquiry IV. Repeat this process with leaves from the plant kept in the dark, being careful not to expose the leaves to light. If it can be arranged, this entire second transfer should be done in a darkened room.

10. Open the jars, place the coleus plants in the jars with the rest of the material used in the experiment and discard in the radioactive waste can.

11. After 24 hours, develop the autoradiograms and examine them.

### Analysis of Data

What does the autoradiogram indicate about the uptake of carbon dioxide by the plant? How does the autoradiogram of the leaf of the plant kept in the light compare with the one of the leaf of the plant kept in the dark? How do you account for this? Is photosynthesis apparently taking place both in the light and in the dark? What does the autoradiogram indicate about the sites of photosynthesis? What does this Inquiry tell you about the source of the carbon used in photosynthesis?

## Problems

This experimental design can be altered to use different plants, different times of exposure, and different plant parts. What would you expect to be the condition of the stem or roots of these plants? If roots absorb $CO_2$, what must be done to the above experimental design to insure that only the leaves and stems are exposed to carbon dioxide?

Test the leaves removed from the coleus plant with the end-window of the G-M tube. In this case, count both upper and lower surfaces of the leaves. Are the experimental results consistent for both the upper and lower surfaces?

A quantitative measure of the count can be obtained by taking a 1-cm square of leaf surface and exposing it to the end-window of the G-M tube.

Can you think of other ways in which radioactive compounds could be used to help explain the processes of photosynthesis?

# ON YOUR OWN

The initial 12 Inquiries have given you some understanding of radiation and its use in biology. You have mastered some techniques for handling sources of radiation and are now able to identify and measure certain types of ionizing radiation. You have become familiar with some of the basic techniques and experimental designs for the use of radionuclides. You are now familiar with the use of radionuclides as tracers and some effects of radiation on certain organisms and their processes. You have seen how radionuclides can be used for a spectrum of problems ranging from those pertaining to microbiology to those that are ecological in nature.

The following suggestions offer opportunities for individual work in addition to the problems in the individual inquiries. They are completely unstructured and are open to a wide variety of variations on a single experimental design and to the testing of a wide variety of hypotheses. For each, you will have to develop a hypothesis, an experimental design, and materials lists that should be checked with your instructor before you proceed. As time and facilities are always a factor, this checking will insure that your design is feasible within the limits of your situation.

1. How Are Radionuclides Used To Measure Volumes In Living Systems?

Radionuclides have a considerable use as tools in determining volumes in living systems that are otherwise difficult or impossible to determine by direct measurement. For example, frequently biologists are asked how much blood there is in a given animal. One way to determine this, of course, is to draw the blood from the animal concerned and measure its volume. However, it is extremely difficult to draw blood from all the tiny capillaries in the body and impossible to do so without the destruction of the animal. A simple, quicker, more accurate technique to answer questions concerning blood volume is through the use of radionuclides.

Blood volume studies are based on the equation

$$V/S = CU/CD$$

V is the animal's total blood volume that we are trying to determine. S is the volume of radionuclide mixture to be injected in the animal (use 0.5 ml for this example). CU (for Counts Undiluted) is the counts per minute of 0.5 ml of S. CD (for Counts Diluted) is the counts per minute of 0.5 ml of the animal's blood withdrawn 5 minutes after the animal is injected with 0.5 ml of the radionuclide mixture. Five-tenths ml of the radionuclide mixture injected into the tail vein of a rat or other mammal will be diluted in proportion to the total blood volume of the animal after about five minutes. When making the counts per minute with the Geiger-Muller counter, the samples are first placed in a planchet and evaporated.

What modifications would have to be made for the radionuclide injected into the blood volume to be recovered in the urine? What radionuclide would you use for this purpose? One could be used that would simply be diluted in the blood plasma. Would this provide an accurate measure of blood volume? Other nuclides can be incorporated into the red blood cells. Would this then give a measure of blood volume? In your experimental design, select the liquid volume to be determined and insure that the above formula will be applicable. Select a proper radionuclide to make the measurements you are seeking. Be sure the experimental design will provide you with data capable of analysis.

* * *

The following three inquiries require a high-energy radiation source. For this purpose, you must be able to enlist the aid of some local physician, dentist, hospital, university, or research center to attain access to x-ray or other irradiation equipment. Arrangements should be made well in advance. Make sure your teacher is involved in such arrangements in order to avoid difficulties with those who operate the radiation sources.

2. What Effects Does Irradiation Have On Metabolism?

Studies of living organisms show that the metabolic rate of the entire organism varies at different times and in different places. Different tissues metabolize at different rates. It is not difficult for you to answer the question as to whether or not an active muscle or piece of bone has a higher metabolic rate. What relationship,

if any, exists between radiation and the metabolic rate of different tissues?

For an experimental design to provide an answer to this question, one must first have access to a radiation source of sufficient intensity to produce radiation capable of tissue damage. Secondly, there needs to be a living organism that is easily obtainable and one whose tissue metabolism is fairly well known. An ideal animal for this source is the common planarian—*Dugesia*. It is small, easily obtainable, and the effects of radiation damage can be observed readily. Expose the planarians to various dosages of irradiation. After irradiation, make careful daily observations of its effects on the planarians.

3. Can Irradiation Act As A Preservative?

Food preservation has been a problem since man first produced more food than he could eat at one time. He was then in a position of trying to preserve this surplus for the next meal or to tide him over the hard winter. Drying and salting were undoubtedly two of the first ways of preserving food. They were followed by pickling, canning, and nowadays, freezing. Today, many of the fresh foods eaten are really fresh frozen foods. Freezing, however, is an expensive technique requiring a constant supply of electrical energy, not only for the initial freezing, but for maintenance of food in the frozen state. In the event of a major power failure over an extended period of time, frozen foods would have to be eaten immediately or spoilage would result. Thus, we have not yet, after many centuries, found the ideal way to preserve food with fresh taste and nutrition intact.

As you know, food spoilage is the result of the multiplication of microorganisms, primarily bacteria or fungi. Theoretically, this multiplication can be stopped by killing all the microorganisms in the food and preventing further contamination. What problems can you suggest that would make such an apparently simple technique so difficult to achieve? Here again, the permanent preservation of food, often called sterilization, requires large amounts of energy. More frequently, food is preserved simply by extending spoiling time, which gives the food a longer shelf life.

You may have heard of the use of high-intensity irradiation as a food preservation technique. For this experimental design, ground meat is a good food to use. By its color and odor, it gives an easy indication of spoilage and, at room temperature, tends to spoil relatively rapidly. Using ground meat, develop an experimental design that will expose the food to high intensity radiation and allow you to measure the degree of preservation of the food so exposed. Would you use thick or thin preparations of food to be preserved by irradiation? How do you prevent subsequent contamination of the irradiated food? Will the food itself be made radioactive by exposure to high intensity radiation? Your experimental design should be so structured to provide answers to these and other similar questions.

4. What Types Of Genetic Damage Are Caused By Irradiation?

Frequently you hear of mutations that are occasioned by exposure to radiation. From your study of evolution, you recognize that mutations are one of the devices whereby change is introduced into the hereditary material. The change may be good, bad, or indifferent. However, as most organisms are fairly well adapted to their environment, a random change is more likely to render them less fit rather than more fit.

If a mutation is a change in a gene, it would be difficult to make a direct observation of a gene change in a chromosome. Such change could only be shown by observing the offspring of parents so irradiated. In Inquiry X, you had an opportunity to see some of the gross effects of irradiation on seeds. There are also observable chromosomal changes that become apparent after irradiation. These include translocations, nondisjunctions, chromosomal duplications, and the appearance of acentric fragments.

However, because chromosome morphology is relatively complex, for the purposes of this investigation it is easiest to compare chromosomes that have been irradiated with normal, nonirradiated chromosomes from the same organism and notice the differences, without specifically trying to name each change. The important observations will be whether chromosomal effects have been produced or not as the result of irradiation, not so much which types have occurred.

A good source of materials for this Inquiry would be the cells of onion root tips undergoing mitosis. The onion tips will need to be exposed to high-intensity radiation, then fixed and

stained to observe the chromosomes. The experimental design should be such that you can make a statement regarding the effect of irradiation on hereditary materials.

5. How Does Irradiation Affect Chemical Reactions In Organisms?

From previous work in biology, you have come to understand that enzymes acting as organic catalysts are necessary for certain chemical reactions to occur in organisms. Enzymes are also protein, and their biological activity in part depends on their particular structure and specific chemical groups. You may also remember that enzymes are specific; that is, they catalyze a particular type of reaction only. The enzyme pepsin, for example, is specific for splitting proteins into smaller units. By irradiating dry, powdered pepsin with a high-energy radiation source such as 60-cobalt, normally available only in hospitals and research institutions, the effect of such irradiation on enzyme activity can be noted.

Develop an experimental design that will test the effects of irradiation on the activity of pepsin. The design should include the amount of pepsin to be irradiated and irradiation doses to be used. Plans for controls should also be included. Egg albumin is a convenient protein. When placed in capillary glass tubes and heated, it turns to a white solid. Using this technique or cutting egg white into small cubes, determine how the effect of the action of pepsin will be measured. Remember the conditions for optimum enzyme actions, such as *p*H and temperature. Are these accounted for in your experimental design? Include in your design plans for the presentation of data. Will graphs or tables best suit your design?

# APPENDIX I HOW ARE MICROORGANISMS HANDLED?

If your laboratory work so far has included microbiological techniques, this Appendix can be skimmed rapidly simply to refresh in your mind the procedures to be used. If you have not yet mastered microbiological techniques, this section should be read with care, for microorganisms are excellent organisms for radiation studies.

*What is a microorganism?*

A microorganism is not a specific kind of animal or plant, but simply one too small to be seen effectively with the unaided eye. Considered as microorganisms are bacteria, yeast, molds, protozoa, and some algae.

*Methods*

Most of the methods used to study microorganisms in general were first developed to study a particular group—the free-living bacteria, which are the smallest of the microorganisms with which you will normally deal. Because you will be dealing with organisms you cannot see, the techniques of handling and growing microorganisms must be mastered before any constructive laboratory work can be undertaken. The same care shown in handling invisible microorganisms will provide valuable clues for proper handling of invisible radiation. It is essential that you learn the methods by which microorganisms are handled and grown. While these methods may seem complicated at first, actually they are simple to learn and essential for a study of microorganisms. The techniques can be mastered with a little practice.

*Aseptic Techniques*

In dealing with microorganisms, the glassware, needles, loops, and all materials used in handling and growing these organisms must be absolutely clean. This does not mean simply washing in soap and water to free these objects of dirt visible to the naked eye; it also means they must be sterilized to remove or kill all microorganisms present. Use of materials that have no living microorganisms on them is an aseptic technique.

Microorganisms can be removed or killed by using heat, as in an autoclave, by flaming, or by chemical means, such as the use of acids, alkalies, salts, and various other compounds, including alcohol and phenol. The chemicals used in sterilization of glassware and equipment are called disinfectants. Those used on living tissues, such as human skin, are called antiseptics and are not as powerful as the disinfectants. Antiseptics and disinfectants will either kill microorganisms or inhibit their growth.

When you work with microorganisms, all equipment and culture media must be sterilized. However, when microorganisms are transferred from one piece of equipment to another, they are at least briefly exposed to an environment in which there are other unwanted microorganisms. If these other microorganisms are introduced into the colony with which you are working, they are called contaminants. We cannot see them with our eyes so we must see them with our minds. In handling microorganisms you must visualize every way in which contaminants can get into cultures. Then, the cultures must be handled in such a way as to prevent the entrance of contaminants.

*Equipment*

Some of the equipment for working with microorganisms is illustrated in Figure 11. The autoclave is used to sterilize all equipment and media not damaged by heat. A pressure cooker can serve the same purpose. Pipettes are of two types: calibrated glass tubes into which liquids can be drawn and medicine dropper pipettes provided with a rubber bulb at one end to give necessary suction. Do not suck liquids by mouth into pipettes. Always use a bulb so that no liquid enters the mouth. The petri dish comes in two parts. Each part is a flat-bottomed glass dish with a small rim. A culture medium is poured into the smaller half of the dish; the other half serves as a lid to prevent microorganisms in the air from getting onto the culture medium. Glassware used in microbiological investigations can be washed and autoclaved.

Microorganisms are so small that it is almost impossible to handle them individually. The inoculating loop and inoculating needle are simple tools designed to handle clusters of microorganisms. Before using either the loop or needle, sterilize them by passing their tips

through a flame until they are red hot, then allow them to cool in the air before use. The sterile loop tip must not be allowed to touch anything except the material to be handled.

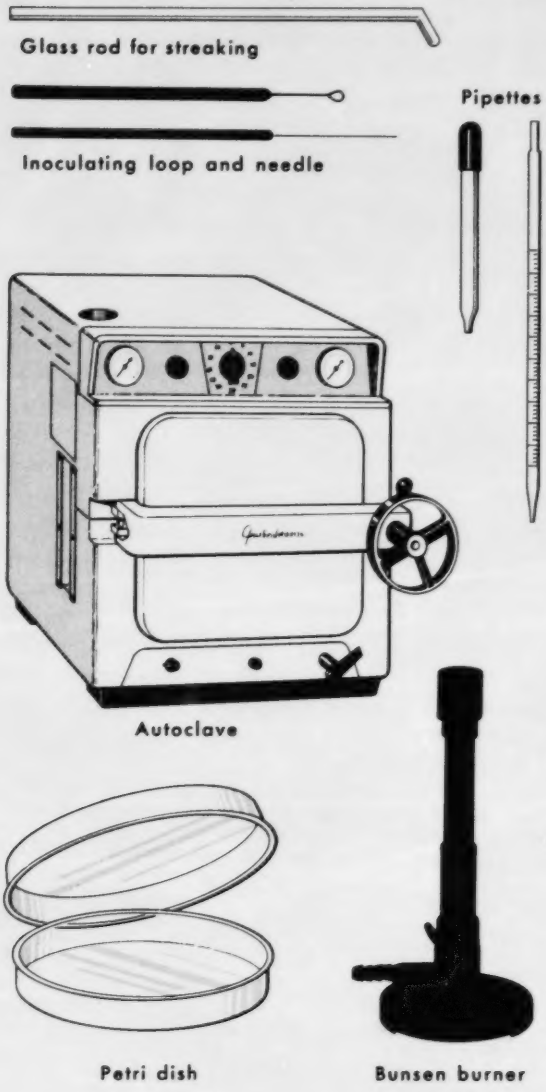



Figure 11. Apparatus necessary for handling microorganisms. A pressure cooker can be substituted for the autoclave.

*Culture Media*

In nature many kinds of microorganisms are found growing together. But, when they are grown or cultured in the laboratory, we usually want to separate the different kinds and grow each separately as a pure culture. One of the big problems in culturing microorganisms is finding a substance on which they can grow.

The technique of separating one species of microorganism from others so that it can be grown in a pure culture was discovered in the laboratory of the German microbiologist, Robert Koch (1843-1910). Koch, as well as several other biologists, discovered that a solid rather than a liquid culture medium could be used to isolate the cultures of microorganisms. As is often the case in science, the studies of Koch and his group were based on the observations of another investigator.

In 1872, Johann Samuel Schroeter noticed that bacteria would grow on the surface of several materials, including that of a cut potato. Here the bacteria formed a large mass which was later called a colony. When the individual colonies were examined under a microscope, each was found to consist of a great number of bacteria. The most important thing was that all the organisms in a single colony were of the same species. Apparently individual cells had landed on the surface of the potato and had then multiplied into millions. These colonies of millions of cells grew large enough to become visible to the naked eye. All the bacteria in a single colony were derived from one cell and hence each colony formed a pure culture.

The potato was not a satisfactory culture medium for a number of reasons. First, it was not an adequate food for all species of microorganisms because only a few kinds would grow on it. Secondly, it was not sterile and contaminants from the knife or from the air would also become attached to its surface and form colonies. Thus, it was not always possible to be sure whether the colonies were from contaminants or were from the microorganisms purposely placed on the potato by the investigator. What was needed was a culture medium that would contain all the substances necessary for growth of bacteria and also be sterile. Koch first used nutrient beef broth soup, which he mixed with gelatin to make it solid. The sterilized mixture was poured into petri dishes to harden. On this surface bacteria were spread, a process called streaking. (Figure 12 shows several different patterns to be used in the streak-plate method.) However, gelatin also has its disadvantages. It melts at 28°C, which is below the best temperature for growth of many bacteria. In addition, many microorganisms make an enzyme—gelatinase—which digests gelatin and causes it to liquefy. Whenever the medium liquefies, the

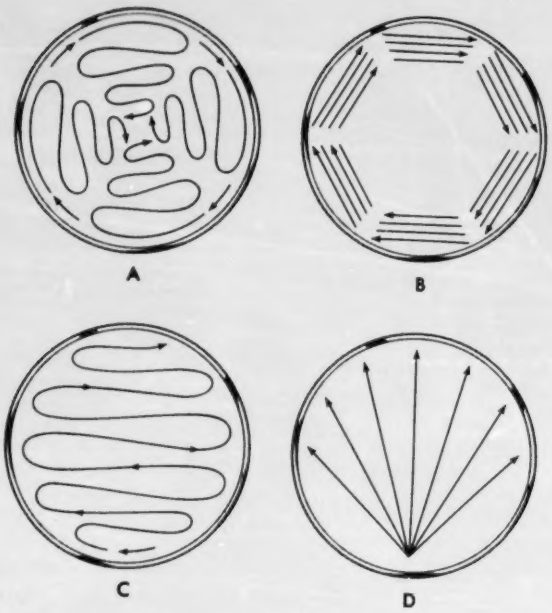



Figure 12. A pattern should be used in streaking microorganisms on agar in petri dishes. The above are four examples of streaking patterns.

separate pure colonies run together and mix.

Agar, an extract of seaweed, proved to be the ideal medium for the solid culture technique. It is attacked by only a few microbial species and it does not soften until a temperature of 100°C is reached. The liquid agar does not solidify until it is cooled to 42°C. The fact that it remains liquid until cooled to this temperature makes it possible to refine methods of obtaining cultures. Thus, instead of using the streaking technique to isolate colonies of bacteria, the culture can be mixed with the cooling agar while the agar is still liquid. The agar-microorganism mixture can then be poured into sterile petri dishes. When the agar solidifies, the individual bacteria are found to be trapped at different locations in the agar, where they develop into separate pure colonies. This technique of obtaining pure bacterial cultures is referred to as the pour-plate method. Figure 13 shows the isolation of bacteria both by the streak-plate method and by the pour-plate method.

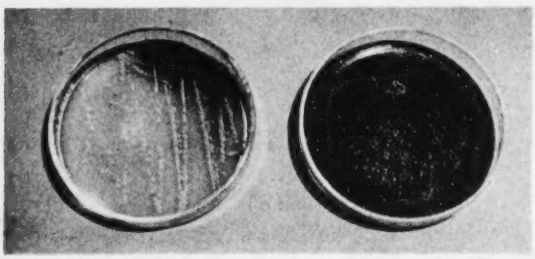


Figure 13. Note the difference in patterns of isolation of bacteria by streaking (left) and use of the pour-plate technique (right). *Photo courtesy of Mrs. Lucille Seguin.*

We still use the pure culture techniques of Koch in microbiological studies and then grow the microorganisms in an incubator at the proper temperature.

*Contamination Prevention*

In any microbial culture the medium must first be freed of foreign organisms by sterilization, and then barriers must be provided to prevent the introduction of contaminants. Cotton has been used to filter out airborne microorganisms since 1855, when a pharmacist used a cotton plug to keep molds out of his drug preparations. Making sterile media, protecting the culture containers with cotton plugs, and using sterilized glassware are necessary for growing microorganisms.

*Techniques*

In handling microorganisms you must be careful not to distribute them carelessly about the working area. This precaution would be essential if microorganisms which caused disease were being used. Even though you will not be working with disease-producing microorganisms you should learn to treat all cultures as if they were. Develop a steady hand for the careful transfer of microbial growth. Do not leave a trail of microorganisms behind you. Sterilize the inoculating loop and needle after you have used them and before putting them down. Put all cultures and other materials containing living microorganisms in containers to be sterilized. Use disinfectant solutions to clean work areas. These practices are designed for everyone's welfare. The same types of careful techniques must be used in handling radioactive materials.

It is essential that you practice the techniques to become skilled at working with microorganisms before you actually begin using them. Competence in handling microorganisms will make your later work in this Block with radionuclides much easier and greatly reduce possibilities of accidents due to careless handling.

Your teacher will demonstrate how to handle microorganisms. Watch the techniques carefully and repeat them until you make no mistakes.

# APPENDIX II *WHAT IS RADIATION?*

In order to understand the phenomenon of radiation and some of its properties, it is necessary to know something about matter, energy, and radionuclides. This Appendix provides some of the background you will find valuable in answering certain of your questions about radiation. All of it is not essential for performance of the work of this Block. Some of the terms that are discussed here are used in the Block, and knowledge of them will be helpful to you.

*Basic Units of Matter*

All matter is made up of three basic units—*protons, neutrons,* and *electrons.* Because neutrons and protons are ordinarily in the nucleus of an atom they are referred to as nucleons (Figure 14). There are many other kinds of atomic particles that have been demonstrated experimentally. You may have read of positrons, mesons, and hyperons, among others. But these are beyond the scope of this Block and they will be neither used nor defined in connection with it. It is necessary only for us to deal with the three fundamental units. Knowledge of their behavior makes the phenomenon of radioactivity understandable.

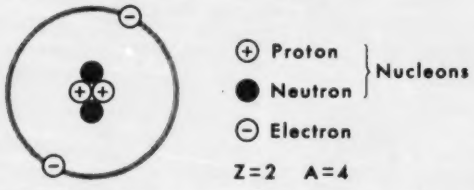



Figure 14. A helium atom made up of protons, neutrons, and electrons. The protons and neutrons together in the nucleus are referred to as nucleons.

*Mass and Energy*

The mass of protons and neutrons is approximately 1800 times that of an electron. (Actually protons are 1836.13 times the mass of an electron, and neutrons have a mass of 1838.13 times that of an electron.) To many people, mass is weight. Before Einstein's statement of the theory of relativity it was assumed that the mass of a body was independent of its motion. However, the variation of mass with speed (velocity) was determined experimentally as early as 1906, using high-speed electrons emitted by radioactive substances. Without going into detail, the general feature of this relationship is that the mass tends to increase as maximum velocity is reached (Table 1).

TABLE 1—INCREASE OF ELECTRON MASS WITH INCREASING VELOCITY

| Velocity of Electron Expressed as a Fraction of Velocity of Light | Relative Mass of Electron |
|---|---|
| At rest | 1.000 |
| 0.3742 | 1.078 |
| 0.5025 | 1.157 |
| 0.6954 | 1.392 |
| 0.8629 | 1.979 |
| 0.9988 | 20.58 |
| 1.0000 (Velocity of light) | Infinite |

Table 1. Mass tends to increase with velocity. Prior to the theory of relativity, it was assumed that the mass of a body remained constant regardless of its motion.

It was to account for this that Einstein introduced the idea that change of mass with changed velocity is equivalent to a change in the energy of the system. This, of course, led to the conclusion that mass is a form of energy. Thus, mass and energy can be expressed in the same unit. This idea is stated in the familiar equation $E = mc^2$. In this equation, energy (E) is equal to the mass (m) times a conversion factor, in this case the square of the speed of light ($c^2$).

If a process could be devised whereby one gram of mass could be converted into energy, such as electrical energy, we would be dealing with a very large amount of energy indeed. One gram of mass would convert into 25,000,000 kilowatt hours of energy. The average home uses about 4,000 kilowatt hours of electrical energy per year. One gram of mass, then, would be enough to supply 6,250 homes with all the needed electrical energy for a period of one year. It is this fact—that a given unit of mass contains huge amounts of energy—that accounts for the force of an atom bomb or makes possible the generation of power from nuclear fission.

*Electrical and Nuclear Forces*

Not only are the masses of the units that make up an atom different, but their electrical charges are different as well. Neutrons have no charge. Protons are positively charged and electrons negatively charged (Figure 14). Protons and electrons have equal but opposite charges. It is these charges that give rise to an electrostatic force. Unlike charges attract each other, and like charges repel. Thus, protons and electrons attract each other, but one proton repels another, and one electron repels another electron. This concept of attraction and repulsion can be demonstrated by using bar magnets as an analogous model. Suspend a bar magnet by a string so that it is free to rotate in any direction. Bring a second bar magnet close to it to demonstrate the effect of attraction and repulsion of like and unlike charges when different poles are used.

It is not possible to demonstrate easily in class but, despite the fact that neutrons possess no electrical charge, when two neutrons are brought very close together they do attract one another. This is not an electrical force. It is known as a *nuclear force*. Nuclear force is reduced with increasing distance and is not noted at distances slightly greater than $10^{-13}$ centimeters. This nuclear force also exists when neutrons and protons or two protons are brought very close together. Normally when two protons are separated by relatively large distances, their electrical charges tend to repel one another. However, when the distances are very short, the nuclear force overcomes the electrical repulsion and they are attracted to each other. Electrical forces vary inversely as the square of the distance between the charged particles. Nuclear forces vary much more rapidly but not according to an accurately known law of variation.

*Atomic Structure, Nuclides, and Isotopes*

We can use the above introduction to the three fundamental units of matter to consider atoms. *Atoms* are the basic building blocks of chemical elements. They are indivisible by chemical means. They are made up of protons, neutrons, and electrons. Their nuclear and electrical forces are important when we consider the energy they contain. The simplest atom that we can imagine is that of common hydrogen (Figure 15). It consists of a single proton around which one electron revolves. All other atoms consist of a compact *nucleus* containing a specific number of protons and neutrons, and surrounding it, a specific number of electrons revolving in orbits (Figure 16). Figure 16 is a more accurate representation of the atom but it is difficult to use as a model. As an introductory model, Figure 15, known as the Bohr model, allows us to make a number of generalizations.

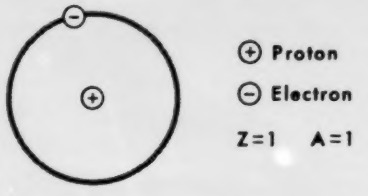



Figure 15. A hydrogen atom. It consists of a nucleus of one proton and one orbital electron. This Bohr model makes it look as if the electron spins around the nucleus in a single plane.

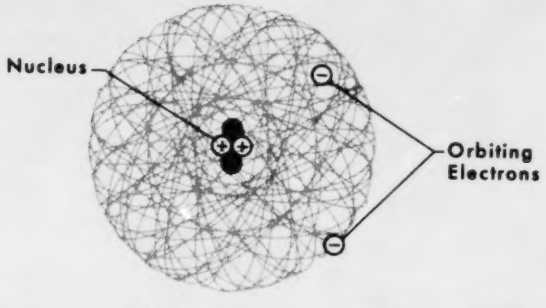



Figure 16. The electrons orbiting an atomic nucleus spin around in a wide variety of planes that seem to form a cloud around the nucleus. Compare this more accurate representation of a helium atom with that of Figure 14, which is a Bohr model of the same atom.

It is nuclear force that binds protons and neutrons in the nucleus and maintains the electrons in closed orbits around the nucleus. Since the atom, as a whole, is electrically neutral, the number of positively charged protons equals the number of negatively charged electrons. The number of protons in an atom is known as the atomic number ($Z$) (Figures 14 and 15). As the atom is normally electrically neutral, if the $Z$ of an atom is 3, what would you expect its number of electrons to be? Another useful number is the *mass number* ($A$), which is the sum of the protons and neutrons in an atom (Figures 14 and 15). It is possible to have atoms with the same atomic number but different mass numbers. These atoms are called *nuclides*. Nuclide is a term generally applied to all atomic forms of the elements. They are distinguished

by their atomic number, mass number, and energy state. The name "nuclide" is often wrongly used in place of the term *isotope*, which applies to two or more nuclides having the same atomic number. Isotope has a more limited meaning and is a term that we do not use generally in this Block. Isotopes are the various forms of a single element and thus are a particular grouping of nuclides. Nuclides, on the other hand, include all the isotopic forms of all the elements. All isotopes of the same element have the same atomic number but differ in mass number, that is, they have the same number of protons but different numbers of neutrons. An unstable isotope of an element that emits radiation is termed a *radioisotope*, of which more than 1300 natural and artificial types have been identified. The term *radionuclide* indicates a radioactive nuclide.

*Atomic Size*

All atoms are built on a plan similar to that of the hydrogen atom. The $Z$ number of protons and the neutrons ($A$ minus $Z$) form the compact nucleus. The electrons circle the nucleus in orbits of varying radii. As $Z$ increases, the size of the nucleus increases. One might then expect the atom to become larger and larger. However, such is not the case. As $Z$ increases, the electrons are attracted more strongly to the nucleus and their orbits decrease in size. Thus, the overall effect of increasing nuclear size and decreasing orbital size is to maintain an almost constant size of atoms, from hydrogen to uranium. The diameter of the largest atom is only about 4 times that of the smallest (Figure 17).

*Atomic Energy*

An idea of how much energy is trapped in an atom can be gained if we first deal with distance. Atoms are mainly empty space. If, for example, the nucleus of a hydrogen atom were a ball 4 inches in diameter, the electron would be a third of a mile away. (Actually, the electron orbit of a hydrogen atom has a radius of $0.53 \times 10^{-8}$ cm and does not approach the nucleus closer than this distance.) Because of the electrostatic force between the electron and the proton, it takes energy to remove the electron. The very smallest amount of energy required to remove the electron from a hydrogen atom is 13.54 electron volts (ev). (An electron volt is the amount of kinetic energy gained by an electron accelerated through an electrical potential difference of one volt. It is a unit of energy for work, not of voltage.)

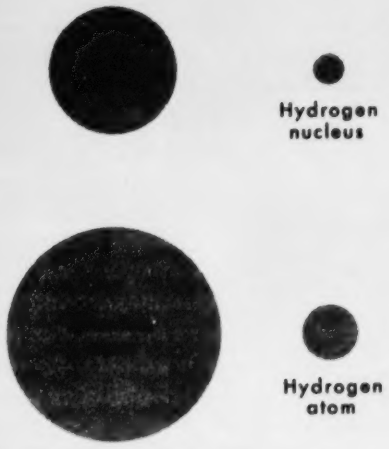



Figure 17. The number of protons in an atomic nucleus ($Z$) varies from one in hydrogen to 92 in uranium. Despite this fact, the uranium atom is not 92 times as large as the hydrogen atom because, while the nuclear size increases, the diameters of the electron orbits decrease so that the difference between the largest atom and the smallest is only about 4 times.

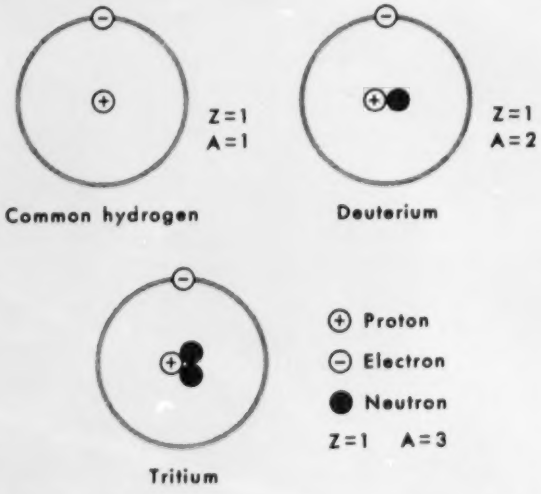



Figure 18. The atomic number ($Z$) of these three forms of hydrogen is the same. The mass number ($A$), which includes both protons and neutrons, varies from one to three. These three forms of the element hydrogen are referred to as isotopes. They have almost the same chemical properties but somewhat different physical properties. Isotopes are the various forms of a single element. The term nuclide is used to refer to all the isotopic forms of all the elements. Thus, isotopes of a single element can be considered to be a family of nuclides.

Atomic energy relationships may be demonstrated in a deuterium atom, which is a nuclide of hydrogen known as heavy hydrogen (Figure 18). It differs from ordinary hydrogen only in

that a neutron is attached to the proton in the nucleus. The atomic number is 1 but the mass number is 2. The electron is relatively unaffected because there is no appreciable force between a neutron and an electron. Because the electron orbits are almost the same in atoms which are nuclides of one another, this applies generally to all nuclides. In the deuterium atom only 13.54 electron volts of energy are required to overcome the electrostatic force between the electron and the proton. However, the nuclear force is so great that about 2.2 million electron volts (2.2 Mev) are needed to break apart the neutron and the proton in the deuterium nucleus. The energy needed to separate the neutron from the proton is called *binding energy*.

Just as the energy required to separate neutrons from protons is called binding energy, so the energy required to break molecules into completely separate atoms is called binding energy. The amount of energy needed to separate carbon from oxygen in a carbon monoxide molecule is about 10 electron volts. How does this compare to the binding energy required to separate a neutron from a proton in a deuterium nucleus?

Naturally occurring atoms have atomic numbers ($Z$) ranging from 1, as in hydrogen, to 92, as in uranium. In an earlier stage in the evolution of the universe, atoms of higher $Z$ than 92 probably existed and may still exist in such environments as the interior of the sun. For most of these $Z$ values, limited numbers of stable nuclides exist.

*Attractive Forces*

What has been said so far indicates that an atom possesses no net electrical charge. However, there are attractive forces between two of these "neutral" atoms at any particular instant due to an unequal distribution of electrical charges in the electrons circling the atom which create electrostatic fields. Thus, when the positive charges are in one position in one atom and the negative charges in an opposite position in another, a temporary attractive force can exist between atoms (Figure 19). There are atomic forces that exist between atoms which are weakly attractive in many cases. These are known as *van der Waals forces*. At much smaller distances, frequently the forces become more strongly attractive. This larger attractive force is a *chemical force* or *chemical bond*. It is

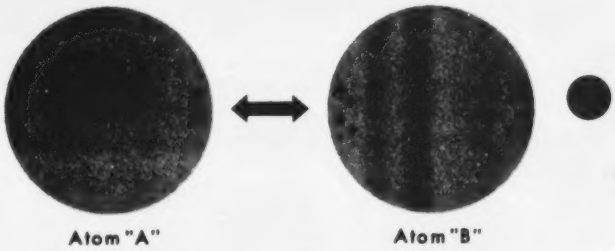



Figure 19. While atoms are normally considered to possess no net electrical charge, unequal distribution of electrical charges due to position of the electrons, as shown in atoms A and B above, may result in a temporary attractive force.

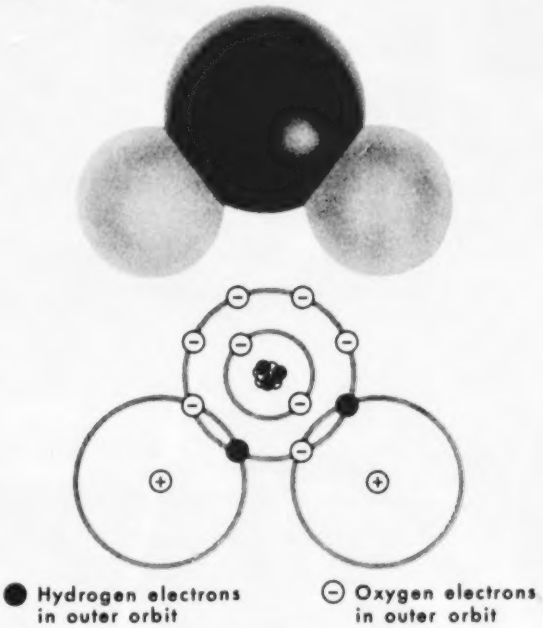



Figure 20. Two representations of the water molecule. In the Stuart model, above, hydrogen atoms are bonded to a single oxygen atom. In the Bohr diagram, below, each hydrogen atom is seen to share its electron with the oxygen and also to share one of the oxygen's electrons. This sharing of electrons bonds the atoms together. The bond is covalent.

this attractive chemical force between atoms that causes them to combine to form *molecules*, which are clusters of two or more atoms (Figure 20).

Apart from their greater strength, chemical forces differ from van der Waals forces in exhibiting a saturation effect that allows a particular atom to be able to combine with only a limited number of other atoms at any one time. A saturated molecule is one in which all the chemical attracting powers of the atoms of which it is composed are used so that the molecule has no chemical attraction for another of

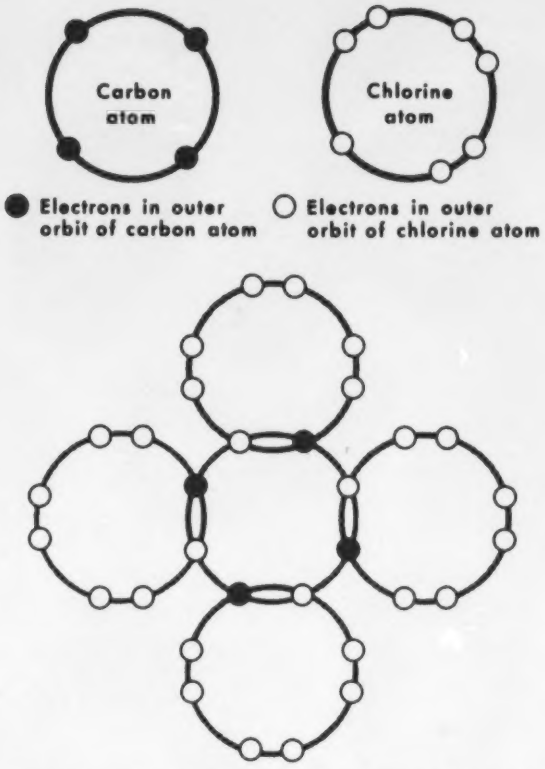



Figure 21. In the carbon tetrachloride molecule all the chemical attracting powers of the carbon atom are used so that there are no more electrons to share. The molecule is said to be saturated.

the same kind. In carbon tetrachloride (Figure 21), four chlorine atoms combine with one atom of carbon. In this molecule, usually none of the atoms has any further chemical attraction for any others.

*Nuclear Reactions*

Just as we have chemical reactions between atoms and molecules, we may have rearrangements of nucleons between atomic nuclei. These reactions are spoken of as nuclear reactions. When we compare a nuclear reaction to a chemical reaction, the energy released in a nuclear reaction is much higher than that released in a chemical reaction. The reason for this is that the binding energy of nucleons in a nucleus is about a million times greater than that of atoms in molecules. If a uranium nucleus breaks up into two nearly equal parts, about 150 million electron volts (150 Mev) of energy is released. Contrast this with the 5 electron volts of energy released when two hydrogen atoms combine with one of oxygen to form a molecule of water in a standard chemical reaction.

*Fission*

Remember that mass is also a form of energy. Since the sum of the masses of the two fragments of the uranium nucleus is less than the mass of the original nucleus, the difference is released as 150 Mev of energy. This breakup of nuclei with the release of energy occurs in the process of *nuclear fission*. Nuclear fission usually takes place in nuclei of mass numbers greater than 56.

*Fusion*

In those elements with nuclei of mass numbers of less than 56, energy is released when certain nuclei are formed from lighter ones. If, for example, helium nuclei could be formed from protons and neutrons, the sum of the masses of these nucleons is greater than the mass of the newly formed nucleus. The difference in mass is released in the form of 28 Mev of energy per helium nucleus formed. This process resulting in nuclear energy release is known as *fusion*. It is the process that is responsible for the heat of the sun and of the stars. The use of the $E = mc^2$ formula shows that the mass of a proton is equivalent to 940 Mev of energy. The mass of a uranium nucleus is 238 times greater.

*Radioactivity and Radionuclides*

Certain atomic nuclei are unstable and break up spontaneously. These are said to be radioactive and include such naturally occurring elements as potassium, rubidium, samarium, lutetium, polonium, radium, radon, thorium, and uranium. When radioactive nuclei release their energy, they do so in the form of alpha or beta particles, or gamma radiations, or a combination of any of these three. For example, 131-iodine emits both gamma rays and beta particles, 32-phosphorus only beta particles, and 210-polonium alpha particles. These radionuclides are written as $^{131}I$, $^{32}P$, and $^{210}Po$. The letters are the chemical symbols for the elements concerned. The numbers are the mass numbers ($A$), the sums of the protons and neutrons in the nucleus. A species of atom that is identified by the number of neutrons and protons in its nucleus, as well as the energy state of the nucleus, is a nuclide. There are over 320 naturally occurring nuclides and almost 900 that have been artificially produced. Nuclides are written with a subscript atomic number ($Z$) preceding the chemical symbol and a superscript mass

number ($A$) preceding it. For example, calcium has an atomic number of 20 and a mass number of 40. This nuclide would be written $^{40}_{20}Ca$.

*Ions and Ionization*

Molecules can be broken up into electrically charged fragments. These fragments of molecules or more complex units are called *ions*. An ion can be an atom or a group of atoms that carries either a positive or a negative electrical charge. Positive ions are formed when electrons are lost. Negative ions are formed when electrons are gained.

When a substance contains ions it is *ionized*. Radiation from radionuclides can break up molecules into ions. This radiation is called *ionizing radiation*. The cloud chamber operates on this principle. The charged particles passing through the gas of the cloud chamber leave a trail of positive and negative ions around which vapor condenses. Both ionized gases and liquids are conductors.

*Half-Life*

Radionuclides emit ionizing radiation. Any emission causes the nucleus of that particular nuclide to change to that of another. Thus, each atom can emit its characteristic radiation only once. The entire sample of radioactive material will ultimately cease its radioactivity in time. It is possible to predict mathematically the decrease in the radioactivity of any given radioactive sample. Scientists commonly use the time for the rate to fall to one-half the initial rate. This value is the *half-life* of the radionuclide. The decrease in radioactivity over a period of time is spoken of as the *decay rate*. The half-life of radionuclides varies greatly. For 45-calcium, it is 165 days; for 14-carbon, 5730 years; for 210-polonium, 138 days; and for 211-polonium, it is 0.52 seconds. Table 2 shows half-lives for some typical radionuclides. Figure 22 shows a generalized decay graph plotting decreased radioactivity against time expressed as half-lives. After five half-lives, how much radioactivity remains in the individual sample? What would be some of the advantages of a radionuclide with a long half-life? With a short half-life? Which type would you use for a long-term experiment?

*Particles and Rays*

Alpha and beta emissions are not a part of the electromagnetic spectrum, but are particles.

TABLE 2—HALF-LIVES OF REPRESENTATIVE RADIONUCLIDES AND THEIR MAJOR RADIATIONS

| Radionuclide | Half-Life | Radiation |
|---|---|---|
| 131-Barium | 12 days | gamma |
| 199-Bismuth | 24 minutes | alpha |
| 14-Carbon | 5730 years | beta |
| 137-Cesium | 30 years | beta, gamma |
| 140-Cesium | 66 seconds | beta |
| 60-Cobalt | 5.25 years | beta, gamma |
| 60-Copper | 23 minutes | beta,gamma |
| 3-Hydrogen | 12 years | beta |
| 131-Iodine | 8.04 days | beta, gamma |
| 210-Lead | 21 years | beta, gamma |
| 27-Magnesium | 9.6 minutes | beta, gamma |
| 16-Nitrogen | 7.3 seconds | beta, gamma |
| 32-Phosphorus | 14.3 days | beta |
| 206-Polonium | 9 days | alpha, gamma |
| 209-Polonium | 103 years | alpha |
| 38-Potassium | 7.5 minutes | beta, gamma |
| 222-Radium | 38 seconds | alpha |
| 225-Radium | 14.8 days | beta |
| 226-Radium | 1600 years | alpha, gamma |
| 22-Sodium | 2.6 years | beta, gamma |
| 90-Strontium | 28 years | beta |
| 35-Sulphur | 88 days | beta |
| 230-Uranium | 20.8 days | alpha |
| 234-Uranium | $2.48 \times 10^5$ years | alpha, gamma |
| 238-Uranium | $4.498 \times 10^9$ years | alpha, gamma |
| 65-Zinc | 244 days | beta, gamma |

Table 2. Can you determine a relationship between elements and half-life or between type of radiation and either half-life or element?

Alpha particles are helium nuclei. They are emitted from radioactive material usually at energies of between 3 to 8 million electron volts. They are positively charged and relatively large. They travel at a speed less than 1/10 the velocity of light.

Beta particles are electrons 1/1837 the mass of a hydrogen atom with energies ranging from a few thousand to a few million electron volts

traveling at speeds of from 25 to 99 percent of the velocity of light.

Gamma rays are short wavelengths of electromagnetic radiation emitted as small packets (photons). They travel at the speed of light. Some characteristics of these radiations are summarized in Table 3. The symbols for alpha and beta particles and gamma radiation are the Greek symbols: Alpha $\alpha$, Beta $\beta$, and Gamma $\gamma$. You will see the biological significance of these three types of radiation as you perform the Inquiries in this Block.

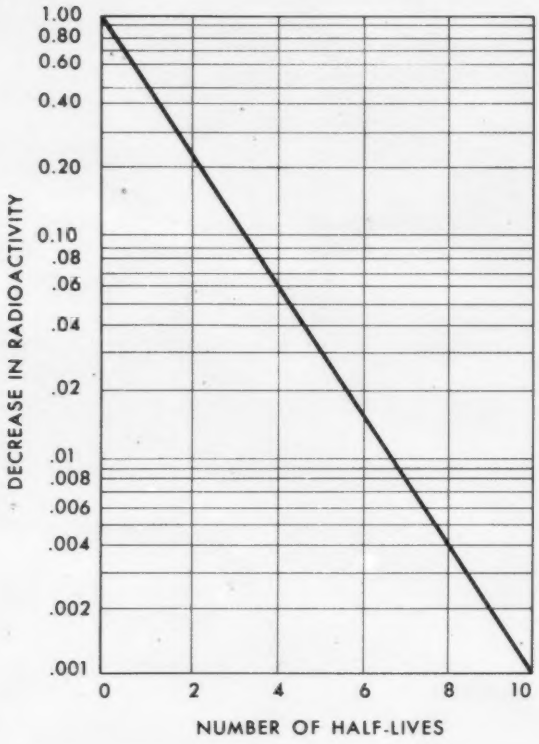



Figure 22. Decrease in radioactivity plotted against half-life. The time for half-life varies with different elements from fractions of seconds to thousands of years. This generalized graph applies to all radioactive materials. After ten half-lives, the radioactivity is reduced to a thousandth of its original intensity. Will it ever reach zero?

*Radiation Units*

Radiation is measured in curies (c). A curie is a relatively large unit of activity of a radionuclide which is equal to $3.7 \times 10^{10}$ radioactive disintegrations per second. A microcurie is a more convenient unit. It is one millionth of a curie and is equal to $3.7 \times 10^{4}$ radioactive disintegrations per second.

In biological work, there are three radiation units that are commonly used. They are as follows:

1. The *roentgen* (r) is a unit of exposure to x or gamma radiation based on the ionizations that these radiations produce in air. An exposure of 1 roentgen results in $2.584 \times 10^{-4}$ coulomb (a unit of electric charge) per kilogram of air. This unit is not applied to particulate radiation, such as that of alpha, beta, or neutron particles. Exposure rate is usually expressed as roentgens per unit of time, roentgens per hour, per minute, per second, etc.
2. The *rad* is a way of expressing the energy absorbed per gram of materials from any ion-

TABLE 3—PROPERTIES OF RADIATION

| | RADIATION | CHARGE | APPROXIMATE ENERGY RANGE | APPROXIMATE RANGE | | PRIMARY SOURCE OF RADIATION |
|---|---|---|---|---|---|---|
| | | | | AIR | WATER | |
| PARTICLES | Alpha | +2 | 3-9 Mev | 2-8 cm | 20-40 $\mu$ | Heavy nuclei |
| | Beta | -1 | 0-3 Mev | 0-10 m | 0-1 mm | Nuclei with high n/p ratio |
| | Neutrons | 0 | 0-10 Mev | 0-100 m | 0-1 m | Nuclear bombardment |
| ELECTROMAGNETIC | X-rays | None | ev-100 Kev | mm-10 m | $\mu$-cm | Orbital electron transitions |
| | Gamma rays | None | 10 Kev-10 Mev | cm-100 m | mm-10 cm | Nuclear transitions |

Table 3. X rays and gamma rays are part of the electromagnetic spectrum. Alpha and beta particles are produced by radioactive elements. Neutrons can be released by use of such devices as high-energy accelerators.

izing radiation. One rad is 100 ergs (a unit of work) absorbed per gram of any substance. The rad is a relatively recent term. A roentgen is a much more common term, particularly in the older literature. In most cases, the number of rads is approximately equal to the number of roentgens. The rad is used for particulate as well as electromagnetic radiation.

3. The *rem* is a unit of dose equivalent. This is numerically equal to the dose in rads multiplied by the proper modifying factors (rbe, q.f., or d.f.—see below).

There are three factors to be considered in radiation measurement. They are as follows:

1. *Relative Biological Effectiveness* (rbe). This term is limited to use in radiobiology to express relative effectiveness of various types of radiations. The rbe values are experimentally determined and pertain only to the system under study.
2. The *quality factor* (q.f.) relates the effect of radiations to that of gamma rays from 60-cobalt. It makes allowances for the fact that the same dose in rads from different types of radiation does not necessarily produce the same biological effect. It is used primarily for the purposes of radiation protection. If gamma rays have a quality factor of 1, alpha particles have a quality factor of 10.
3. The dose *distribution factor* (d.f.) is used to calculate the rem when considering internally deposited radionuclides. It takes into account the nonuniform distribution of a given radionuclide in various organs in the body.

# APPENDIX III *HOW ARE RADIOACTIVE MATERIALS PRODUCED?*

When particles are emitted from an atomic nucleus, the atom is changed. Through these changes it becomes an atom of a different element. The alchemists of ancient times sought vainly to change (transmute) lead into gold. Now we can perform such transmutations, but the cost of so doing is far greater than the value of the elements produced. As an example of transmutation let's first consider an alpha particle. It consists of two protons and two neutrons. It has an atomic number of 2 and a mass number of 4. Any nucleus which emits an alpha particle changes into a nucleus with a mass number less by four and an atomic number less by two than that of the original element. Thus, polonium with a mass number of 210 and an atomic number of 84 consists of 126 neutrons and 84 protons. The element resulting after the emission of an alpha particle from polonium is lead with a mass number of 206 and an atomic number of 82 (Figure 23).

Another example is provided in a study by Rutherford in 1919 concerning alpha particles and nitrogen. This work showed that if an alpha particle hit a nitrogen nucleus, its large kinetic energy (K.E.) of 7.7 Mev (million electron volts) overcame the repulsion of the nuclear charges. A highly unstable compound nucleus of a rare nuclide of flourine was then formed. This nuclide disintegrates immediately to release a high-K.E. proton and a stable nucleus of a rare nuclide of oxygen (Figure 24). This was the first example of an artificially produced transmutation.

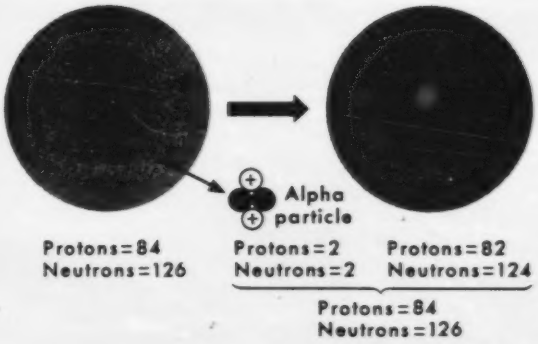



Figure 23. Polonium can be changed to lead by emission of an alpha particle, which changes both the mass number ($A$) and the atomic number ($Z$).

Nuclear reactions can be written as formulae the same way as can nonnuclear chemical reactions. The summary of the helium-nitrogen reaction above is as follows:

$$^{4}_{2}\mathrm{He} + {}^{14}_{7}\mathrm{N} \longrightarrow {}^{1}_{1}\mathrm{H} + {}^{17}_{8}\mathrm{O}$$

This equation balances. The sum of the atomic numbers (nuclear charges or position in the periodic table) is the same on both sides of the reaction (+9). The sums of the mass numbers (18) are also the same. Since the intermediate flourine nucleus disappears immediately, it is usually omitted from the written reaction.

Similarly, if we split a single electron from the nucleus of a lead nuclide with an atomic number of 82 and a mass number of 210, a bis-

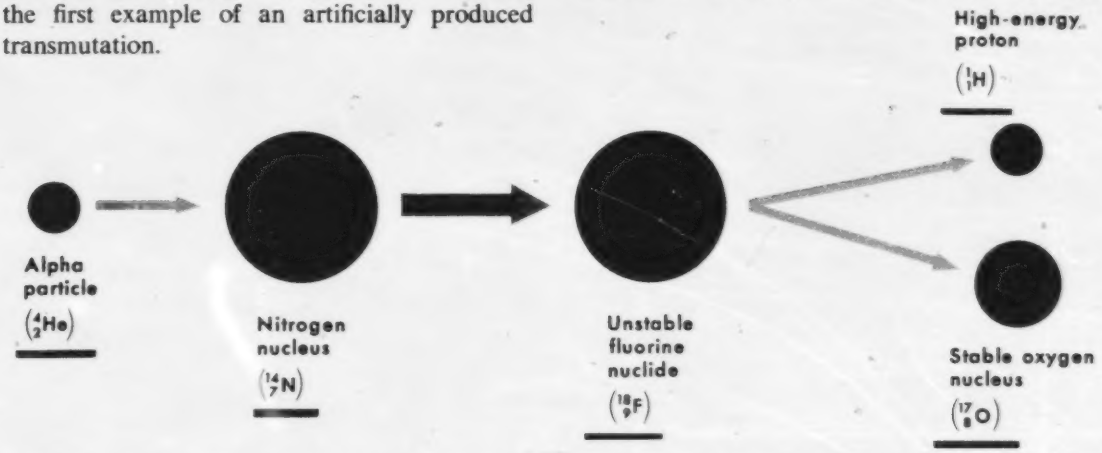



Figure 24. The first laboratory-produced transmutation occurred in 1919, when a nitrogen nucleus was used as a target for an alpha particle. The nitrogen nucleus changes into a rare nuclide of flourine, which rapidly breaks down to form oxygen with the release of a high-energy proton.

muth nuclide is formed with a mass number of 210 and an atomic number of 83 (Figure 25). The emission of a beta particle—an electron—by a nucleus increases the atomic number by one. It does not change the mass number since the mass of an electron is small. The reason the atomic number increases by one is that it is assumed a neutron in the nucleus splits into a proton and an electron. The proton remains in the nucleus and the electron is ejected from it. Thus, the mass remains unchanged but the number of neutrons is decreased by one and the number of protons is increased by one.

*Radionuclides*

Radionuclides can be manufactured when a neutron, proton, alpha particle, deuteron, or gamma ray from outside an atom reaches its nucleus and interacts with its nucleons. When 31-phosphorus captures a neutron in its nucleus it becomes the radionuclide 32-phosphorus

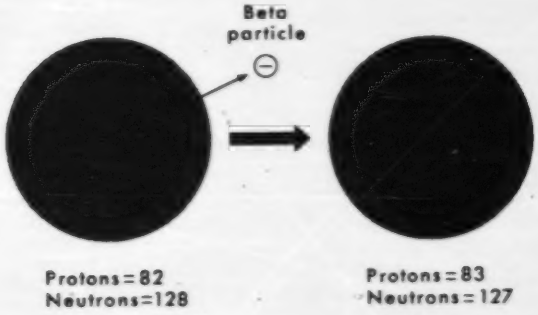



Figure 25. Transmutations can occur not only by emission of alpha particles, but also by the emission of a beta particle—an electron. The loss of the electron increases the atomic number by one and results in the production of bismuth from lead in this example.

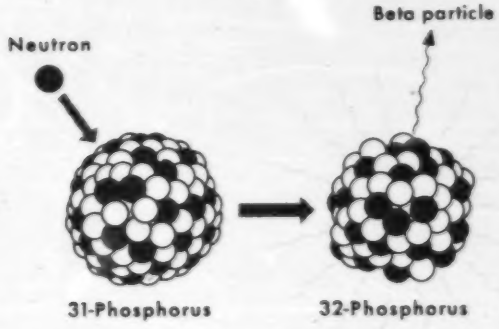
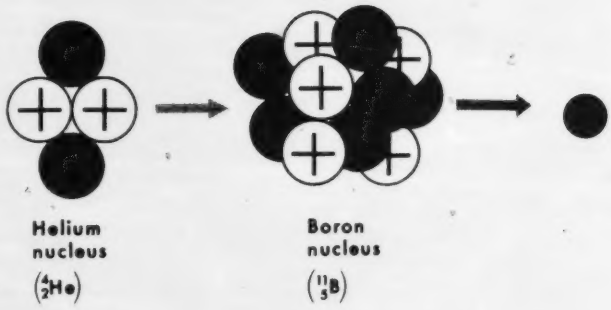
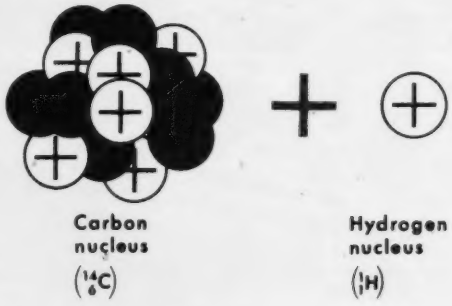



Figure 26. Radionuclides can be manufactured when an atom is bombarded by neutrons. In this case, stable phosphorus absorbing a neutron becomes radioactive 32-phosphorus. This type of radioactivity can be produced in a nuclear reactor.




Boron nucleus (5 protons, 6 neutrons) about to capture helium nucleus (2 protons, 2 neutrons).




Boron nucleus bombarded by helium nucleus becomes radioactive carbon (6 protons, 8 neutrons) plus one hydrogen nucleus.

Figure 27. Bombarding nuclei with alpha particles (helium nuclei) also causes transmutation. In this case, boron is changed to radioactive carbon with an emission of a proton (hydrogen nucleus).

and emits beta particles. The original stable target nucleus has been transformed into a radioactive one (Figure 26). This type of nuclear reaction also can be shown when the nucleus of the element boron is bombarded by an alpha particle (helium nucleus with two protons and two neutrons). It is transformed into a 14-carbon nucleus and releases a proton (hydrogen nucleus) (Figure 27).

*Accelerators*

Charged particles such as alpha particles or protons must have a very high energy of motion in order to reach the interior of a target nucleus. They must overcome being repulsed by the electrical charge of the nucleus. Neutrons are normally used because, being uncharged, they do not show this repulsion effect. The lack of charge is of value in penetrating the nucleus, but neutrons cannot be accelerated by electrical fields because of this very lack of charge. They are, however, released with considerable energy in certain nuclear reactions. In a mixture of

plutonium and beryllium, the plutonium emits alpha particles. These react with the beryllium, which then releases neutrons. These can be used as target material to hit other nuclei. The beryllium itself is transformed into 12-carbon (Figure 28).

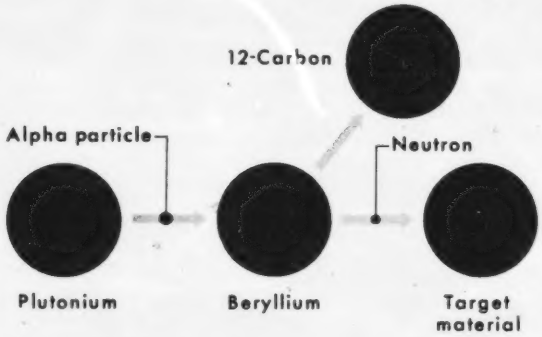



Figure 28. Neutrons are generated from a mixture of plutonium and beryllium. The alpha particle from the plutonium reacts with the beryllium nucleus, which changes into 12-carbon and emits a neutron that can then be used to hit the nucleus of a given target element.

It is possible to use alpha particles from natural sources to bombard nuclei. A far greater variety of nuclear reactions, however, can be brought about with beams of charged particles from such accelerators as cyclotrons, betatrons, synchrotrons, linear accelerators, and similar devices. In these machines, ions are speeded up and directed by a combination of electric and magnetic fields under vacuum conditions. As many as 31 billion electron volts (Bev) have been given to protons in these devices. Nuclear reactions in accelerators are accompanied by release of energy. But this is not the way to generate energy commercially, because, like the situation of turning lead to gold, more energy has to be used up to produce the reaction than can be released. Therefore, these instruments are basically for research.

*Chain Reactions*

Fission with uranium is a chain reaction. Naturally occurring uranium consists of two nuclides. One is of the mass 238 ($^{238}U$) and is the far more abundant. The other ($^{235}U$) is present in only 1/140 part of $^{238}U$. A slow neutron is readily captured on impact with the $^{235}U$. This capture creates a new nucleus of the same atomic number but with its mass number increased by one. Thus, $^{236}U$ is formed. $^{236}U$ is an unstable element and undergoes fission which releases a few more neutrons, usually two or three. These neutrons then are captured by other nuclei of $^{235}U$, which split and release more neutrons. Thus a chain reaction is set up (Figure 29). A large mass of uranium can be fissioned with great generation of energy, about 20,000 kilowatt hours per gram of $^{235}U$. One condition necessary for the chain reaction is a sufficiently large mass of uranium. If only a small volume of uranium is available, most of the neutrons escape from the small mass without producing fission. In a larger mass, however, most of the neutrons are captured by the nuclei and relatively few escape. This is a critical mass —one of such size that fission occurs within it. With natural uranium alone, it would not be possible to set up a chain reaction because the $^{238}U$, which is most abundant, absorbs neutrons without undergoing fission. By artificial nuclide

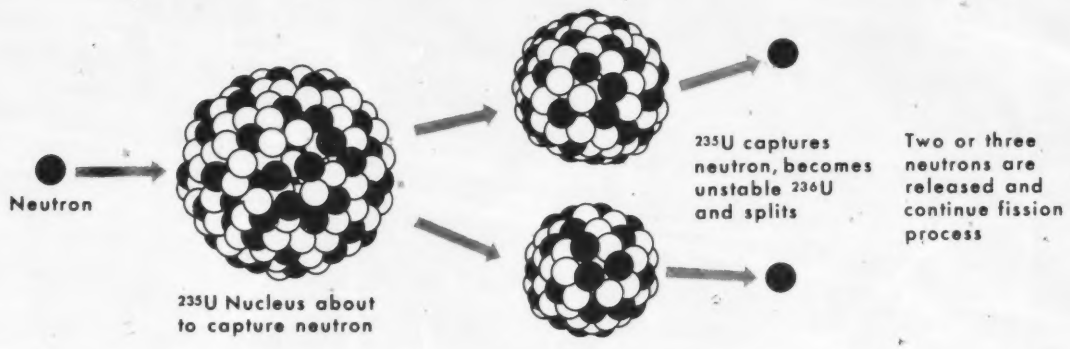

Neutron
235U Nucleus about to capture neutron
235U captures neutron, becomes unstable 236U and splits
Two or three neutrons are released and continue fission process

Figure 29. Fission occurs when a heavy nucleus is split into two approximately equal parts to form nuclei of lighter elements. This splitting is accompanied by the release of a large amount of energy and one or more neutrons that continue the chain reaction.

separation in our laboratories, relatively pure $^{235}U$ can be produced and chain reactions may be initiated within a mass of critical size or larger. This can be used either in an atomic bomb or in reactors to produce energy that could be used for constructive purposes.

*Nuclear Reactors*

A pile of uranium of critical mass or larger that includes devices whereby the energy of motion of neutrons is reduced is called a nuclear reactor. The energy absorber in this case is spoken of as a moderator. The moderator is usually graphite or heavy water (water in which hydrogen is replaced by the nuclide deuterium). If the uranium is sufficiently rich in $^{235}U$, ordinary water can be used as a moderator. As the neutrons become slowed by the moderators, they are not absorbed appreciably by $^{238}U$. Thus, the moderator slows down neutrons. But enough still escapes absorption by $^{238}U$ to maintain the chain reaction. In a nuclear reactor, the rate of generation of energy is controlled by insertion of adjustable rods of materials such as boron or cadmium, which are strong neutron absorbers.

The nuclear reactor generates power through its heat emission. It also creates radionuclides for use in various ways, because nuclear fragments resulting from fission are unstable. It is possible to create radioactive nuclides of different elements by inserting the natural element into the reactor so that it captures neutrons and becomes radioactive. It is these radionuclides that we will be using in this Laboratory Block.

*Use of Radionuclides—Tracers*

Radionuclides have a wide range of application in industry, agriculture, medicine, and research. It is important to remember that the radionuclide of an element behaves chemically the same way as does the normal element. However, because it is radioactive, it can be followed through biological processes even when present in very small quantities. Because it can be followed in this fashion, a radionuclide can be referred to as a tracer. In addition to use of radionuclides as tracers, they can be used to induce mutations, diagnose disease, treat certain kinds of diseases such as cancer, preserve food, and eliminate insect pests. What other uses of radionuclides occur to you?

# APPENDIX IV *HOW DO YOU OPERATE A GEIGER-MULLER COUNTER?*

Inquiries III and IV provide data on two radiation detection devices—the cloud chamber and photographic film. Various other devices, including proportional and scintillation counters, are used for the immediate detection of radiation and its measurement. One of the most common devices is the Geiger-Muller (G-M) tube and its accompanying measuring instrument (scaler or rate meter). Together, the tube and the measuring instrument are called a counter.

The structure and operation of the Geiger-Muller tube are essentially simple. An understanding of its operation is necessary in order to use it to make meaningful determinations of radioactivity. The tube itself is basically a pair of electrodes separated by an easily ionizable gas. The tube case can act as the negative electrode; the center wire is the positive one. A high electrical potential is maintained between the two electrodes. When radiation ionizes the gas, the ions thus produced travel to the electrodes. The motion of these ions produces an electrical current detected in the tube and recorded by either a scaler or a rate meter.

A simplified diagram of a Geiger-Muller tube is shown in Figure 30. This is a typical end-window type of tube closed by a thin window at one end. There are also side-window tubes not illustrated. Both are filled with either helium or argon gas. These are called counting gases. In operation, electrons travel to the positive anode. The positively charged ions travel toward the negative cathode. Both acquire energy. These particles produce still other ions when they collide with the gas molecules. This

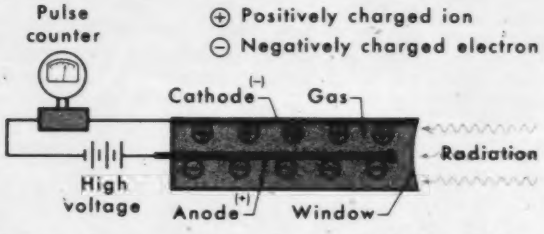



Figure 30. A diagram of an end-window Geiger-Muller tube. The gas consists of a counting gas, such as helium or argon, and a quenching gas, such as butane or chlorine. The window is very thin and should never be touched. To prevent breakage, it is protected by a metal shield.

is almost like an ion chain reaction. If there were no way of controlling it, one ionization would cause the tube to discharge continuously.

One way to prevent this from happening is by placing a quenching gas in the tube. The quenching gas absorbs some of the energy of the electrons and positive ions. If the quenching gas is alcohol or butane, the tube is said to be organically quenched. These molecules of quenching gas are decomposed so that the tube has a useful life of about $10^8$ counts (about 20 months of counting at the rate of 1000 counts per minute). Halogen-quenched tubes utilize bromine, chlorine, or some other similar molecules as quenching agents. They have a much longer life because atoms of these quenching gases recombine.

Tubes that use these gases are said to be internally quenched. Another way to prevent continuous discharge is to remove the high voltage from the tube momentarily. This can be done electronically. The tube is then said to be externally quenched.

Most Geiger-Muller tubes have certain characteristics in common that affect their counting ability. There are four major factors that influence the efficiency of the tube. They are:

1. *Resolving time.* After ionization has begun, there are short periods of time when the tube will not register additional ions. These periods constitute the resolving time. During resolving time, two or more ionizing particles will be counted as one. Because of this, the number of counts recorded will always be less than the actual number of particles or rays passing into the tube. The maximum number of radiation events that can be registered by a single tube amounts to about 50,000 counts per minute.

2. *Absorption factor.* All particles or rays emitted from a radioactive source are not detected. Some of the radiation is absorbed by the sample itself, some by the air between the sample and the tube, and some by the window of the Geiger-Muller tube. For the work in this Laboratory Block, the absorption factor is considered insignificant.

3. *Counting geometry.* This term refers to the physical relationship (position, distance,

holder, etc.) between the radiation source and the detecting equipment (Geiger-Muller tube). It commonly expresses the percent of radiation reaching the detection equipment from the radioactive source. The closer the radioactive source to the tube the greater the percent of emitted radiation detected. For comparable results the same counting geometry should be maintained when making a number of readings.

4. *Scattering.* This is the process by which a particle's path is changed. Scattering may be due to the mass of the sample itself, the air between the radiation source and the tube, or by the wall of the tube shield. When particles are directed back toward the counter, we speak of backscattering. The degree of backscattering varies with the energy of the radiation, atomic number, and the thickness of the support materials. It may increase the count by as much as 50 percent.

In addition to this background information, your teacher will demonstrate the use of the Geiger-Muller counter to you. Follow the written directions as the counter is being demonstrated. You may be asked to plot a curve of the operating characteristics of the counter. If so, prepare a graph with counts per minute on the vertical axis from 0 to 6,000 in 1000's. The horizontal axis should be for voltage. Begin with 800 volts and increase by 100's to 1300 volts. You may have to change these figures depending on the operating characteristics of your counter.

*Cautions*

Before beginning to use a Geiger-Muller counter, the following cautions should be observed:

1. The high voltage carried by the Geiger-Muller tube is dangerous. The tube and its connections should be inspected prior to use for possible defects before high voltage is applied. Instruments should always be handled carefully.
2. The tube window is extremely thin and very easily broken. Under no conditions should it be touched. Damage to the window renders the tube useless. To prevent such damage, three operational procedures should be followed. Keep the shield over the window when the tube is handled. Remove it only when directed to do so. Mount the tube in a vertical position and bring the specimens to it rather than using the tube as a moving probe.
3. Operating at too high a voltage is the cause of most failures of detection equipment. Dependable operation requires a proper voltage setting. The tube can be permanently damaged by an increase in voltage. Never exceed the voltage setting recommended for your instrument.

Just as there are various kinds of microscopes in different biology classrooms, so there may be different scalers or rate meters to which a Geiger-Muller tube can be attached. However, the principles are similar for all and the following generalized description can be modified as necessary:

1. Always read the operating manual for the particular instrument you are to use. Learn the location and use of each of the controls and understand the scale divisions of the meter. Should any questions arise at any point in the operation of the Geiger-Muller counter, always check with your instructor before proceeding.
2. Make sure the chassis of the scaler or rate meter is grounded if the line cord does not have a grounding connection.
3. Before plugging the meter cord into a 110-volt, 60-cycle outlet, check to see that the master power switch is off. Check to be sure that the high-voltage switch is off. Turn it as far counterclockwise as it will go, which sets the voltage as low as possible.
4. Make sure that the Geiger-Muller tube is connected to the proper jack on the scaler. The anode connects to the positive terminal of the high-voltage supply. The cathode connects to the negative terminal, which is normally the chassis ground.
5. Turn the master switch on and allow the instrument to warm up. In vacuum tube instruments, this may take from 20 to 30 minutes. For normal classroom use, the master switch is not turned off unless the instrument is to be used less than twice a week. Most instruments have a pilot light to indicate when the power is on.
6. After the instrument has warmed up, turn the high-voltage switch on. Do not increase the voltage beyond the minimal setting for 20 to 30 minutes. This not only protects the electronics, but stabilizes the high voltage

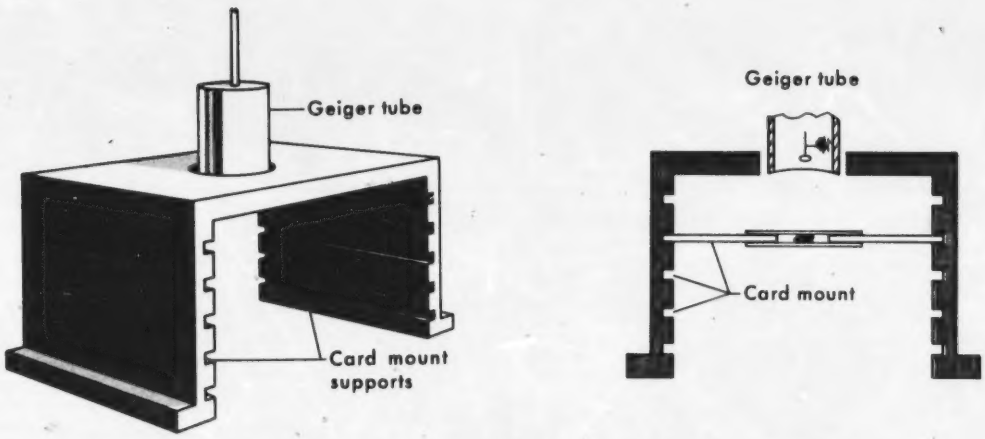



Figure 31. To preserve counting geometry, a special holder for the Geiger-Muller tube and mounted radioactive samples may be used.

power supply so that future readings will be consistent.

7. Place the Geiger-Muller tube with the end window down in a special holder (Figure 31) or secure it by means of a clamp to a ring stand (Figure 32). Place a card mount containing the sample on a counting shelf or on a piece of glass beneath the Geiger-Muller tube. Uncover the window, turn the count switch to the count position, and slowly increase the high voltage until the scaler begins to indicate a count.

Plot the point on your graph where the scaler begins to indicate a count. This is the starting potential for the G-M tube.

8. Increase the voltage in 50-volt steps and take a count at each step. As radiation is a sequence of random events, the longer the counting period, the better. Short periods tend to be unreliable. Count for at least 5-minute periods at each voltage setting. Good technique calls for taking the average from three or more such counting periods. Plot each of these points on your graph.

9. Note the point where an increase in voltage increases the count very little. This is the beginning of the plateau. The point is called the threshold potential of the tube. What is it for the tube you are using? In determining the extent of the plateau, note when a further small increase in voltage begins to cause a rapid increase in count rate. At this point turn down the voltage at once! Do not increase it when the graph slope begins to rise from the plateau!

10. In organically quenched tubes, the plateau region usually has a slope of less than 3 percent. For halogen-quenched tubes, the slope is higher. Determining the plateau is important, for it is in this region that the proper operating voltage is selected. The tube should be operated with voltage relatively close to the threshold. Good practice calls for operating voltages within the lower 25 per-

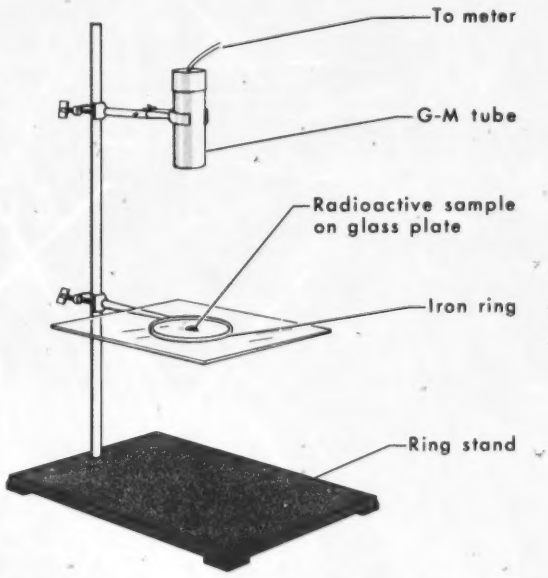



Figure 32. If special holders are not available, the Geiger-Muller tube can be held in a clamp mounted on a ring stand. The sample can be placed on a glass plate and moved toward the tube by adjusting the height of the iron ring. Possible tube damage is minimized by having the tube held steady and by moving the sample rather than by moving the tube toward the sample.

cent of the plateau to conserve the life of the tube. Color this section of your graph. What voltages does it span?

11. After the high voltage is properly adjusted, the count switch should be turned to stop and the reset switch depressed. The scaler is now ready for operation. All that is necessary for operation now is to turn the high voltage switch to *on* and the count switch to *count*.

12. Your teacher may wish to demonstrate the consequences of raised voltage. If the voltage is increased above that for the plateau region, the tube begins to discharge continuously and may be seriously damaged. Whenever count-rate increases rapidly above that of the plateau region, the voltage should be turned down immediately to prevent tube damage. It is necessary to estimate count-rate at high voltages rather than to damage the tube by attempting to obtain measurements at such levels. If by accident the voltage is set too high, the scaler jams. The only action to take is to turn the voltage down instantly! Switching the count switch to stop does not cut off the high voltage to the tube!

13. Having plotted the counts per minute on graph paper, compare your graph with one that the teacher will place on the board. You have now determined the threshold and operating plateau for the Geiger-Muller counter you will use. Remember that voltage for subsequent experiments must be set in the lower 25 percent of plateau operating range. Tube characteristics change with age and use. With long usage, it is necessary periodically to redetermine the characteristic curve for the tube being used. However, for the short time the Geiger-Muller counter will be used in this Block, this step will not be necessary.

*Analysis of Data*

What are the first steps in preparing to use a Geiger-Muller counter? What precautions must be observed in its operation? At what voltage should it be operated? What is the greatest single source of danger to the counter?

To what uses might the counter be put? Contrast the counter with the cloud chamber and the film badge as a detection and measurement instrument. Of what value is a counter to a biologist?

## SELECTED REFERENCES ON RADIATION

The Atomic Energy Commission (AEC) has produced a large number of printed materials, some of which are available free upon request in small numbers. The "Understanding the Atom" series contains over 40 titles. A single copy of any one booklet in this series, or of no more than three different booklets, will be sent free upon request. Teachers and librarians can obtain complete sets if requests are on school letterhead and their proposed use indicated.

Requests relative to nuclear science can also be directed to the AEC if the exact topic of interest is stated and the reason for the request is presented.

The address for both requests and publications is:

United States Atomic Energy Commission
Division of Technical Information
Extension
Educational Materials Section
P. O. Box 62
Oak Ridge, Tennessee 37830

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